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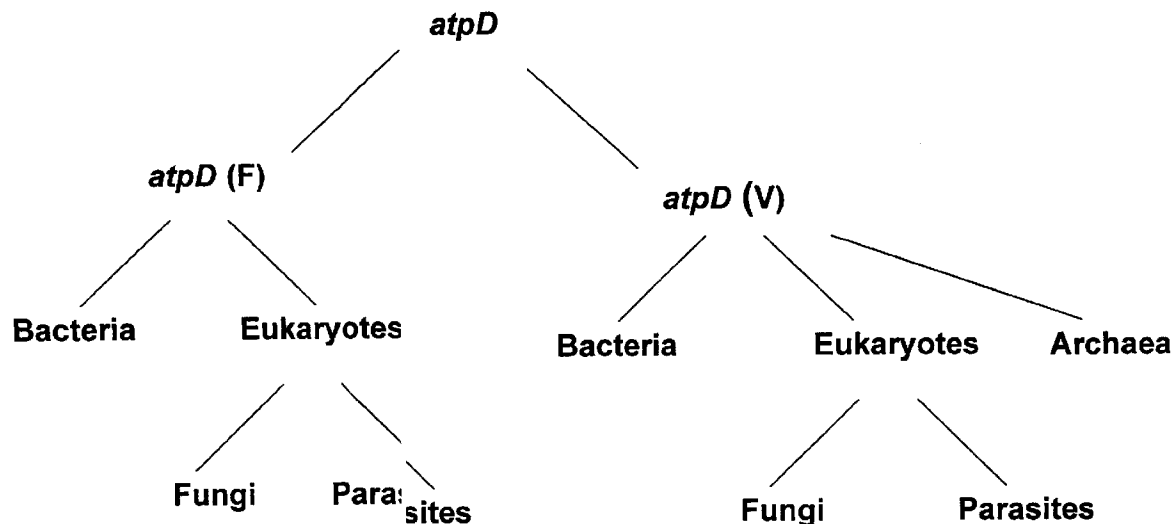
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(54) **Titre : ACIDES NUCLEIQUES ET METHODES DE DETECTION DE STREPTOCOQUE**

(54) **Title: NUCLEIC ACIDS AND METHODS FOR THE DETECTION OF STREPTOCOCCUS**



(57) **Abrégé/Abstract:**

Four highly conserved genes, encoding translation elongation factor Tu, translation elongation factor G, the catalytic subunit of proton-translocating ATPase and the RecA recombinase, are used to generate a sequence repertory or bank and species-specific, genus-specific, family-specific, group-specific and universal nucleic acid probes and amplification primers to rapidly detect and identify algal, archaeal, bacterial, fungal and parasitical microorganisms from specimens for diagnosis. The detection of associated antimicrobial agents resistance and toxin genes are also under the scope of the present invention.



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ABSTRACT

Nucleic acids, methods and diagnostic kits for detecting in a sample at least one microorganism belonging to the *Streptococcus* genus are provided. The method comprises i) contacting the sample with at least one species-specific or genus-specific oligonucleotide selected to be sufficiently complementary to hybridize to at least one *tuf* nucleic acid specific to the genus; ii) Allowing the oligonucleotide and the *tuf* nucleic acid to hybridize under conditions such that the oligonucleotide hybridizes to the *tuf* nucleic acid, wherein the oligonucleotide hybridizes only to microorganisms belonging to the target *Streptococcus* genus and does not detectably hybridize to *tuf* nucleic acids from non-target microorganisms; and iii) Testing for hybridization of the oligonucleotide to the *tuf* nucleic acid.

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
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TITLE OF THE INVENTION

NUCLEIC ACIDS AND METHODS FOR THE DETECTION OF *STREPTOCOCCUS*

BACKGROUND OF THE INVENTION

Classical methods for the identification of microorganisms

Microorganisms are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, generally two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan™ system from Dade Behring and the Vitek™ system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these

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faster systems always require the primary isolation of the bacteria or fungi as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. So, the shortest time from sample reception to identification of the pathogen is around 24 hours. Moreover, fungi other than yeasts are often difficult or very slow to grow from clinical specimens. Identification must rely on labor-intensive techniques such as direct microscopic examination of the specimens and by direct and/or indirect immunological assays. Cultivation of most parasites is impractical in the clinical laboratory. Hence, microscopic examination of the specimen, a few immunological tests and clinical symptoms are often the only methods used for an identification that frequently remains presumptive.

The fastest bacterial identification system, the autoSCAN-Walk-Away™ system (Dade Behring) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5 to 6 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than *Enterobacteriaceae* (Croizé J., 1995, Lett. Infectiol. **10**:109-113; York *et al.*, 1992, J. Clin. Microbiol. **30**:2903-2910). For *Enterobacteriaceae*, the percentage of non-conclusive identifications was 2.7 to 11.4%. The list of microorganisms identified by commercial systems based on classical identification methods is given in Table 15.

A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the main organisms associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

Clinical specimens tested in clinical microbiology laboratories

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Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and antibiotic susceptibility.

Conventional pathogen identification from clinical specimens

Urine specimens

The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 μ L of urine is streaked on agar plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial count of 10^7 CFU/L or more in urine. However, infections with less than 10^7 CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. 311:560-564). Importantly, approximately 80% of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10^7 CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

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Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koenig *et al.*, 1992, J. Clin. Microbiol. **30**:342-345; Pezzlo *et al.*, 1992, J. Clin. Microbiol. **30**:640-684).

Blood specimens

The blood specimens received in the microbiology laboratory are always submitted for culture. Blood culture systems may be manual, semi-automated or completely automated. The BACTEC™ system (from Becton Dickinson) and the BacTAlert™ system (from Organon Teknika Corporation) are the two most widely used automated blood culture systems. These systems incubate blood culture bottles under optimal conditions for growth of most bacteria. Bacterial growth is monitored continuously to detect early positives by using highly sensitive bacterial growth detectors. Once growth is detected, a Gram stain is performed directly from the blood culture and then used to inoculate nutrient agar plates. Subsequently, bacterial identification and susceptibility testing are carried out from isolated bacterial colonies with automated systems as described previously. Blood culture bottles are normally reported as negative if no growth is detected after an incubation of 6 to 7 days. Normally, the vast majority of blood cultures are reported negative. For example, the percentage of negative blood cultures at the microbiology laboratory of the CHUL for the period February 1994-January 1995 was 93.1% (Table 3).

Other clinical samples

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Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial, pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3). In all these normally sterile sites, tests for the universal detection of algae, archaea, bacteria, fungi and parasites would be very useful .

Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial or fungal pathogens potentially associated with the infection are grown and separated from the colonizing microbes using selective methods and then identified as described previously. Of course, the DNA-based universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non-sterile sites. On the other hand, DNA-based assays for species or genus or family or group detection and identification as well as for the detection of antimicrobial agents resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

DNA-based assays with any specimen

There is an obvious need for rapid and accurate diagnostic tests for the detection and identification of algae, archaea, bacteria, fungi and parasites directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Bergeron and Ouellette, 1995, Infection 23:69-72; Bergeron and Ouellette, 1998, J Clin Microbiol. 36:2169-72). The DNA probes and amplification primers which are objects of the present invention are applicable for the detection and identification of algae, archaea, bacteria, fungi, and parasites directly from any clinical specimen such as blood,

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urine, sputum, cerebrospinal fluid, pus, genital and gastro-intestinal tracts, skin or any other type of specimens (Table 3). These assays are also applicable to detection from microbial cultures (e.g. blood cultures, bacterial or fungal colonies on nutrient agar, or liquid cell cultures in nutrient broth). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since these tests can be performed in one hour or less, they provide the clinician with new diagnostic tools which should contribute to a better management of patients with infectious diseases. Specimens from sources other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock, food products, environment such as water or soil, and others) may also be tested with these assays.

A high percentage of culture-negative specimens

Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of normally sterile clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would also be desirable, in addition to identify bacteria at the species or genus or family or group level in a given specimen, to screen out the high proportion of negative clinical specimens with a DNA-based test detecting the presence of any bacterium (i.e. universal bacterial detection). As disclosed in the present invention, such a screening test may be based on DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for any bacterium would give a positive amplification signal. Similarly, highly conserved genes of fungi and parasites could serve not only to identify particular species or genus or family or group but also to detect the presence of any fungi or parasite in the specimen.

Towards the development of rapid DNA-based diagnostic tests

A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antimicrobial agents resistance genes from clinical samples (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). There is no need for culture of the pathogens, hence the organisms can be detected directly from clinical samples, thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for microbial identification than currently used phenotypic identification systems which are based on biochemical tests and/or microscopic examination. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* as well as for the detection of a variety of viruses (Tang Y. and Persing D. H., Molecular detection and identification of microorganisms, In: P. Murray *et al.*, 1999, Manual of Clinical Microbiology, ASM press, 7th edition, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention, for example: *Staphylococcus* sp. (US patent serial no. 5,437,978), *Neisseria* sp. (US patent serial no. 5,162,199 and European patent serial no. 0,337,896,131) and *Listeria monocytogenes* (US patent serial nos. 5,389,513 and 5,089,386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from those described in the present invention. To our knowledge there are only four patents published by others mentioning the use of

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any of the four highly conserved gene targets described in the present invention for diagnostic purposes (PCT international publication number WO92/03455 and WO00/14274, European patent publication number 0 133 671 B1, and European patent publication number 0 133 288 A2). WO92/03455 is focused on the inhibition of *Candida* species for therapeutic purposes. It describes antisense oligonucleotide probes hybridizing to *Candida* messenger RNA. Two of the numerous mRNA proposed as targets are coding for translation elongation factor 1 (tef1) and the beta subunit of ATPase. DNA amplification or hybridization are not under the scope of their invention and although diagnostic use is briefly mentioned in the body of the application, no specific claim is made regarding diagnostics. WO00/14274 describes the use of bacterial *recA* gene for identification and speciation of bacteria of the *Burkholderia cepacia* complex. Specific claims are made on a method for obtaining nucleotide sequence information for the *recA* gene from the target bacteria and a following comparison with a standard library of nucleotide sequence information (claim 1), and on the use of PCR for amplification of the *recA* gene in a sample of interest (claims 4 to 7, and 13). However, the use of a discriminatory restriction enzyme in a RFLP procedure is essential to fulfill the speciation and WO00/14274 did not mention that multiple *recA* probes could be used simultaneously. Patent EP 0 133 288 A2 describes and claims the use of bacterial *tuf* (and *fus*) sequence for diagnostics based on hybridization of a *tuf* (or *fus*) probe with bacterial DNA. DNA amplification is not under the scope of EP 0 133 288 A2. Nowhere it is mentioned that multiple *tuf* (or *fus*) probes could be used simultaneously. No mention is made regarding speciation using *tuf* (or *fus*) DNA nucleic acids and/or sequences. The sensitivities of the *tuf* hybridizations reported are 1×10^6 bacteria or 1-100 ng of DNA. This is much less sensitive than what is achieved by our assays using nucleic acid amplification technologies.

Although there are phenotypic identification methods which have been used for more than 125 years in clinical microbiology laboratories, these methods do not provide information fast enough to be useful in the initial management of patients.

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There is a need to increase the speed of the diagnosis of commonly encountered bacterial, fungal and parasitical infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the microbial genotype (e.g. DNA level) is more stable than the phenotype (e.g. physiologic level).

Bacteria, fungi and parasites encompass numerous well-known microbial pathogens. Other microorganisms could also be pathogens or associated with human diseases. For example, achlorophyllous algae of the *Prototheca* genus can infect humans. Archae, especially methanogens, are present in the gut flora of humans (Reeve, J.H., 1999, J. Bacteriol. **181**:3613-3617). However, methanogens have been associated to pathologic manifestations in the colon, vagina, and mouth (Belay *et al.*, 1988, Appl. Enviro. Microbiol. **54**:600-603; Belay *et al.*, 1990, J. Clin. Microbiol. **28**:1666-1668; Weaver *et al.*, 1986, Gut **27**:698-704).

In addition to the identification of the infectious agent, it is often desirable to identify harmful toxins and/or to monitor the sensitivity of the microorganism to antimicrobial agents. As revealed in this invention, genetic identification of the microorganism could be performed simultaneously with toxin and antimicrobial agents resistance genes.

Knowledge of the genomic sequences of algal, archaeal, bacterial, fungal and parasitical species continuously increases as testified by the number of sequences available from public databases such as GenBank. From the sequences readily available from those public databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial, fungal and parasitical pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iii) the family-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iv) the group-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (v) the

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universal detection of algal, archaeal, bacterial, fungal or parasitical pathogens, and/or (vi) the specific detection and identification of antimicrobial agents resistance genes, and/or (vii) the specific detection and identification of bacterial toxin genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

In our assigned U.S. patent 6,001,564 and our WO98/20157 patent publication, we described DNA sequences suitable for (i) the species-specific detection and identification of clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of antimicrobial agents resistance genes.

The WO98/20157 patent publication describes proprietary *tuf* DNA sequences as well as *tuf* sequences selected from public databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from these sequences. All the nucleic acid sequences described in that patent publication can enter in the composition of diagnostic kits or products and methods capable of a) detecting the presence of bacteria and fungi b) detecting specifically at the species, genus, family or group levels, the presence of bacteria and fungi and antimicrobial agents resistance genes associated with these pathogens. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and associated antimicrobial agents resistance genes and toxins genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antimicrobial agents resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent applications.

The present invention improves the assigned application by disclosing new proprietary *tuf* nucleic acids and/or sequences as well as describing new ways to

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obtain *tuf* nucleic acids and/or sequences. In addition we disclose new proprietary *atpD* and *recA* nucleic acids and/or sequences. In addition, new uses of *tuf*, *atpD* and *recA* DNA nucleic acids and/or sequences selected from public databases (Table 11) are disclosed.

Highly conserved genes for identification and diagnostics

Highly conserved genes are useful for identification of microorganisms. For bacteria, the most studied genes for identification of microorganisms are the universally conserved ribosomal RNA genes (rRNA). Among those, the principal targets used for identification purposes are the small subunit (SSU) ribosomal 16S rRNA genes (in prokaryotes) and 18S rRNA genes (in eukaryotes) (Relman and Persing, Genotyping Methods for Microbial Identification, *In*: D.H. Persing, 1996, PCR Protocols for Emerging Infectious Diseases, ASM Press, Washington D.C.). The rRNA genes are also the most commonly used targets for universal detection of bacteria (Chen *et al.*, 1988, FEMS Microbiol. Lett. **57**:19-24; McCabe *et al.*, 1999, Mol. Genet. Metabol. **66**:205-211) and fungi (Van Burik *et al.*, 1998, J. Clin. Microbiol. **36**:1169-1175).

However, it may be difficult to discriminate between closely related species when using primers derived from the 16S rRNA. In some instances, 16S rRNA sequence identity may not be sufficient to guarantee species identity (Fox *et al.*, 1992, Int. J. Syst. Bacteriol. **42**:166-170) and it has been shown that inter-operon sequence variation as well as strain to strain variation could undermine the application of 16S rRNA for identification purposes (Clayton *et al.*, 1995, Int. J. Syst. Bacteriol. **45**:595-599). The heat shock proteins (HSP) are another family of very conserved proteins. These ubiquitous proteins in bacteria and eukaryotes are expressed in answer to external stress agents. One of the most described of these HSP is HSP 60. This protein is very conserved at the amino acid level, hence it has been useful for phylogenetic studies. Similar to 16S rRNA, it would be difficult to

discriminate between species using the HSP 60 nucleotide sequences as a diagnostic tool. However, Goh *et al.* identified a highly conserved region flanking a variable region in HSP 60, which led to the design of universal primers amplifying this variable region (Goh *et al.*, US patent serial no. 5,708,160). The sequence variations in the resulting amplicons were found useful for the design of species-specific assays.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

- from any algal, archaeal, bacterial, fungal or parasitical species in any sample suspected of containing said nucleic acids, and optionally,
- from specific microbial species or genera selected from the group consisting of the species or genera listed in Table 4, and optionally,
- from an antimicrobial agents resistance gene selected from the group consisting of the genes listed in Table 5, and optionally,
- from a toxin gene selected from the group consisting of the genes listed in Table 6,

wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probes or primers;

said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any

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microbial species, specific microbial species or genus or family or group and antimicrobial agents resistance gene and/or toxin gene.

In a specific embodiment, a similar method directed to each specific microbial species or genus or family or group detection and identification, antimicrobial agents resistance genes detection, toxin genes detection, and universal bacterial detection, separately, is provided.

In a more specific embodiment, the method makes use of DNA fragments from conserved genes (proprietary sequences and sequences obtained from public databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted algal, archaeal, bacterial, fungal or parasitical nucleic acids.

In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers. To be a good diagnostic candidate, an oligonucleotide of at least 12 nucleotides should be capable of hybridizing with nucleic acids from given microorganism(s), and with substantially all strains and representatives of said microorganism(s); said oligonucleotide being species-, or genus-, or family-, or group-specific or universal.

In another particularly preferred embodiment, oligonucleotides primers and probes of at least 12 nucleotides in length are designed for their specificity and ubiquity based upon analysis of our databases of *tuf*, *atpD* and *recA* sequences. These databases are generated using both proprietary and public sequence information. Altogether, these databases form a sequence repertory useful for the design of primers and probes for the detection and identification of algal, archaeal, bacterial, fungal and parasitical microorganisms. The repertory can also be subdivided into subrepertories for sequence analysis leading to the design of various primers and probes.

The *tuf*, *atpD* and *recA* sequences databases as a product to assist the design of oligonucleotides primers and probes for the detection and identification of algal, archaeal, bacterial, fungal and parasitical microorganisms are also covered.

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The proprietary oligonucleotides (probes and primers) are also another object of this invention.

Diagnostic kits comprising probes or amplification primers such as those for the detection of a microbial species or genus or family or phylum or group selected from the following list consisting of *Abiotrophia adiacens*, *Acinetobacter baumannii*, *Actinomycetae*, *Bacteroides*, *Cytophaga* and *Flexibacter* phylum, *Bacteroides fragilis*, *Bordetella pertussis*, *Bordetella* sp., *Campylobacter jejuni* and *C. coli*, *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *Candida zeylanoides*, *Candida* sp., *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Clostridium* sp., *Corynebacterium* sp., *Cryptococcus neoformans*, *Cryptococcus* sp., *Cryptosporidium parvum*, *Entamoeba* sp., *Enterobacteriaceae* group, *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus* sp., *Escherichia coli* and *Shigella* sp. group, *Gemella* sp., *Giardia* sp., *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Legionella* sp., *Leishmania* sp., *Mycobacteriaceae* family, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, platelets contaminants group (see Table 14), *Pseudomonas aeruginosa*, *Pseudomonads* group, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus* sp., *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus* sp., *Trypanosoma brucei*, *Trypanosoma cruzi*, *Trypanosoma* sp., *Trypanosomatidae* family, are also objects of the present invention.

Diagnostic kits further comprising probes or amplification primers for the detection of an antimicrobial agents resistance gene selected from the group listed in Table 5 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of a toxin gene selected from the group listed in Table 6 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of any other algal, archaeal, bacterial, fungal or parasitical species than those specifically listed herein, comprising or not comprising those for the detection of the specific microbial species or genus or family or group listed above, and further comprising or not comprising probes and primers for the antimicrobial agents resistance genes listed in Table 5, and further comprising or not comprising probes and primers for the toxin genes listed in Table 6 are also objects of this invention.

In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or genus or family or group; or universal detection of algae, archaea, bacteria, fungi or parasites; or antimicrobial agents resistance genes; or toxin genes; or for the detection of any microorganism (algae, archaea, bacteria, fungi or parasites).

In the above methods and kits, probes and primers are not limited to nucleic acids and may include, but are not restricted to analogs of nucleotides such as: inosine, 3-nitropyrrole nucleosides (Nichols *et al.*, 1994, Nature **369**:492-493), Linked Nucleic Acids (LNA) (Koskin *et al.*, 1998, Tetrahedron **54**:3607-3630), and Peptide Nucleic Acids (PNA) (Egholm *et al.*, 1993, Nature **365**:566-568).

In the above methods and kits, amplification reactions may include but are not restricted to: a) polymerase chain reaction (PCR), b) ligase chain reaction (LCR), c) nucleic acid sequence-based amplification (NASBA), d) self-sustained sequence replication (3SR), e) strand displacement amplification (SDA), f) branched DNA signal amplification (bDNA), g) transcription-mediated amplification (TMA), h) cycling probe technology (CPT), i) nested PCR, j) multiplex PCR, k) solid phase amplification (SPA), l) nuclease dependent signal amplification (NDSA), m) rolling circle amplification technology (RCA), n) Anchored strand displacement amplification, o) Solid-phase (immobilized) rolling circle amplification.

In the above methods and kits, detection of the nucleic acids of target genes may include real-time or post-amplification technologies. These detection

technologies can include, but are not limited to, fluorescence resonance energy transfer (FRET)-based methods such as adjacent hybridization to FRET probes (including probe-probe and probe-primer methods), TaqMan, Molecular Beacons, scorpions, nanoparticle probes and Sunrise (Amplifluor). Other detection methods include target genes nucleic acids detection via immunological methods, solid phase hybridization methods on filters, chips or any other solid support, whether the hybridization is monitored by fluorescence, chemiluminescence, potentiometry, mass spectrometry, plasmon resonance, polarimetry, colorimetry, or scanometry. Sequencing, including sequencing by dideoxy termination or sequencing by hybridization, e.g. sequencing using a DNA chip, is another possible method to detect and identify the nucleic acids of target genes.

In a preferred embodiment, a PCR protocol is used for nucleic acid amplification, in diagnostic method as well as in method of construction of a repertory of nucleic acids and deduced sequences.

In a particularly preferred embodiment, a PCR protocol is provided, comprising, an initial denaturation step of 1-3 minutes at 95 °C, followed by an amplification cycle including a denaturation step of one second at 95 °C and an annealing step of 30 seconds at 45-65°C, without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with most selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific, antimicrobial agents resistance gene and toxin gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

It is also an object of the present invention that *tuf*, *atpD* and *recA* sequences could serve as drug targets and these sequences and means to obtain them revealed in the present invention can assist the screening, design and modeling of these drugs.

It is also an object of the present invention that *tuf*, *atpD* and *recA* sequences could serve for vaccine purposes and these sequences and means to obtain them

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revealed in the present invention can assist the screening, design and modeling of these vaccines.

We aim at developing a universal DNA-based test or kit to screen out rapidly samples which are free of algal, archaeal, bacterial, fungal or parasitical cells. This test could be used alone or combined with more specific identification tests to detect and identify the above algal and/or archaeal and/or bacterial and/or fungal and/or parasitical species and/or genera and/or family and/or group and to determine rapidly the bacterial resistance to antibiotics and/or presence of bacterial toxins. Although the sequences from the selected antimicrobial agents resistance genes are available from public databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current diagnostic methods based on bacterial cultures. Using an amplification method for the simultaneous or independent or sequential microbial detection-identification and antimicrobial resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure should save lives by optimizing treatment, should diminish antimicrobial agents resistance because less antibiotics will be prescribed, should reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and side effects of drugs, and decrease the time and costs associated with clinical laboratory testing.

In another embodiment, sequence repertories and ways to obtain them for other gene targets are also an object of this invention, such is the case for the *hexA* nucleic acids and/or sequences of *Streptococci*.

In yet another embodiment, for the detection of mutations associated with antibiotic resistance genes, we built repertories to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. Such repertories and ways to obtain them for *pbp1a*, *pbp2b* and *pbp2x* genes of sensitive and penicillin-resistant *Streptococcus pneumoniae* and also for *gyrA* and

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parC gene fragments from various bacterial species are also an object of the present invention.

The diagnostic kits, primers and probes mentioned above can be used to identify algae, archaea, bacteria, fungi, parasites, antimicrobial agents resistance genes and toxin genes on any type of sample, whether said diagnostic kits, primers and probes are used for *in vitro* or *in situ* applications. The said samples may include but are not limited to: any clinical sample, any environment sample, any microbial culture, any microbial colony, any tissue, and any cell line.

It is also an object of the present invention that said diagnostic kits, primers and probes can be used alone or in conjunction with any other assay suitable to identify microorganisms, including but not limited to: any immunoassay, any enzymatic assay, any biochemical assay, any lysotypic assay, any serological assay, any differential culture medium, any enrichment culture medium, any selective culture medium, any specific assay medium, any identification culture medium, any enumeration culture medium, any cellular stain, any culture on specific cell lines, and any infectivity assay on animals.

In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from proprietary fragments or from public databases. DNA fragments selected from public databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

In another embodiment, the amino acid sequences translated from the repertory of *tuf*, *atpD* and *recA* nucleic acids and/or sequences are also an object of the present invention.

It is clear to the individual skilled in the art that other oligonucleotide sequences appropriate for (i) the universal detection of algae, archaea, bacteria, fungi or parasites, (ii) the detection and identification of the above microbial species or genus or family or group, and (iii) the detection of antimicrobial agents resistance genes, and (iv) the detection of toxin genes, other than those listed in

Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV may also be derived from the proprietary fragments or selected public database sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from public databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific, family-specific, group-specific, resistance gene-specific, toxin gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV which are suitable for diagnostic purposes. When a proprietary fragment or a public databases sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

Since a high percentage of clinical specimens are negative for bacteria (Table 3), DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and public database sequences. The amplification primers were selected from genes highly conserved in algae, archaea, bacteria, fungi and parasites, and are used to detect the presence of any algal, archaeal, bacterial, fungal or parasitical pathogen in clinical specimens in order to determine rapidly whether it is positive or negative for algae,

archaea, bacteria, fungi or parasites. The selected genes, designated *tuf*, *fus*, *atpD* and *recA*, encode respectively 2 proteins (elongation factors Tu and G) involved in the translational process during protein synthesis, a protein (beta subunit) responsible for the catalytic activity of proton pump ATPase and a protein responsible for the homologous recombination of genetic material. The alignments of *tuf*, *atpD* and *recA* sequences used to derive the universal primers include both proprietary and public database sequences. The universal primer strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for microbiological testing.

Table 4 provides a list of the archaeal, bacterial, fungal and parasitical species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are revealed in the present invention. Tables 5 and 6 provide a list of antimicrobial agents resistance genes and toxin genes selected for diagnostic purposes. Table 7 provides the origin of *tuf*, *atpD* and *recA* nucleic acids and/or sequences listed in the sequence listing. Tables 8-10 and 12-14 provide lists of species used to test the specificity, ubiquity and sensitivity of some assays described in the examples. Table 11 provides a list of microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases. Table 15 lists the microorganisms identified by commercial systems. Tables 16-18 are part of Example 42, whereas Tables 19-20 are part of Example 43. Tables 21-22 illustrate Example 44, whereas Tables 23-25 illustrate Example 45.

In accordance with the present invention is provided a method for generating a repertory of nucleic acids of *tuf*, *fus*, *atpD* and/or *recA* genes from which are derived probes or primers, or both, useful for the detection of one, more than one related microorganisms, or substantially all microorganisms of a group selected from algae, archaea, bacteria, fungi and parasites, which comprises the step of:

- amplifying the nucleic acids of a plurality of determined algal, archaeal, bacterial, fungal and parasitical species with any combination of the primer pairs defined in SEQ ID NOs.: 558-561, 562-574, 636-655, 664, 681-683, 696-697, 699-700, 708, 812-815, 911-917, 919-922, 935-938, 1203-1207, 1212-1213, 1221-1229, 1605-1606, 1974-1984, 1999- 2003, 2282-2285.

The terms "related microorganisms" are intended to cover microorganisms that share a common evolutive profile up to the speciation e.g. those that belong to a species, a genus, a family or a phylum. The same terms are also intended to cover a group of different species that are grouped for a specific reason, for example, because they all have a common host tissue or cell. In one specific example, a group of microorganisms potentially found in platelet preparations are grouped together and are considered "related" organisms for the purpose of their simultaneous detection in that particular type of sample.

The repertories *per se* of nucleic acids and of sequences derived therefrom are also provided, as well as "gene banks" comprising these repertories.

For generating sequences of probes or primers, the above method is reproduced or one may start from the sequence repertory or gene bank itself, and the following steps are added:

- aligning a subset of nucleic acid sequences of said repertory,
- locating nucleic acid stretches that are present in the nucleic acids of strains or representatives of said one, more than one related microorganisms, or substantially all microorganisms of said group, and not present in the nucleic acid sequences of other microorganisms, and

deriving consensus nucleic acid sequences useful as probes or primers from said stretches.

Once the sequences of probes or primers are designed, they are converted into real molecules by nucleic acid synthesis.

From the above methods and resulting repertoires, probes and primers for the universal detection of any one of alga, archaeon, bacterium, fungus and parasite are obtainable.

More specifically, the following probes or primers having the sequence defined in SEQ ID NOs.: 543, 556-574, 636-655, 658-661, 664, 681-683, 694, 696, 697, 699, 700, 708, 812-815, 911-917, 919-922, 935-938, 1203-1207, 1212-1213, 1221-1229, 1605-1606, 1974-1984, 1999-2000, 2282-2285 or any variant of at least 12 nucleotides capable of hybridizing with the targeted microorganism(s) and these sequences and a diagnostic method using the same are provided.

Further, probes or primers having specific and ubiquitous properties for the detection and identification of any one of an algal, archaeal, bacterial, fungal and parasitital species, genus, family and group are also designed and derived from the same methods and repertoires.

More specifically, are provided definite probes or primers having specific and ubiquitous properties for the detection and identification of microorganisms.

Indeed, a general method is provided for detecting the presence in a test sample of any microorganism that is an alga, archaeum, bacterium, fungus or parasite, which comprises:

a) putting in contact any test sample *tuf* or *atpD* or *recA* sequences and nucleic acid primers and/or probes, said primers and/or probes having been selected to be sufficiently complementary to hybridize to

one or more *tuf* or *atpD* or *recA* sequences that are specific to said microorganism:

b) allowing the primers and/or probes and any test sample *tuf* or *atpD* or *recA* sequences to hybridize under specified conditions such as said primers and/or probes hybridize to the *tuf* or *atpD* or *recA* sequences of said microorganism and does not delectably hybridize to *tuf* or *atpD* or *recA* sequences from other microorganisms; and,

c) testing for hybridization of said primers and/or probes to any test sample *tuf* or *atpD* or *recA* sequences.

In the latter, step c) is based on a nucleic acid target amplification method, or on a signal amplification method.

The terms "sufficiently complementary" cover perfect and imperfect complementarity.

In addition to the universal or the specific detection and/or identification of microorganisms, the simultaneous detection of antimicrobial agent resistance gene or of a toxin gene is provided in compositions of matter as well as in diagnostic methods. Such detection is brought by using probes or primers having at least 12 nucleotides in length capable of hybridizing with an antimicrobial agent resistance gene and/or toxin gene, a definite set thereof being particularly provided.

Of course, any propriatory nucleic acid and nucleotide sequence derived therefrom, and any variant of at least 12 nucleotides capable of a selective hybridization with the following nucleic acids are within the scope of this invention as well as derived recombinant vectors and hosts:

SEQ ID NOs.: 1-73, 75-241, 399-457, 498-529, 612-618, 621-624, 675, 677, 717-736, 779-792, 840-855, 865, 868-888, 897-910, 932, 967-989, 992, 1266-1297, 1518-1526, 1561-1575, 1578-1580, 1662-1664, 1666-1667, 1669-1670, 1673-1683, 1685-1689, 1786-1843, 1874-1881, 1956-1960, 2183-2185, 2187-2188, 2193-2201, 2214-2249, 2255-2272, which are all *tuf* sequences;

SEO ID NOs.: 242-270, 272-398, 458-497, 530-538, 663, 667, 673-676, 678-680, 737-778, 827-832, 834-839, 856-862, 866-867, 889-896, 929-931, 941-966, 1245-1254, 1256-1265, 1527, 1576-1577, 1600-1604, 1638-1647, 1649-1660, 1671, 1684, 1844-1848, 1849-1865, 2189-2192, which are all *atpD* sequences;

SEQ ID NOs.: 990-991, 1003, 1288-1289, 1714, 1756-1763, 1866-1873 and 2202-2212, which are all *recA* sequences; and

SEQ ID NOs.: 1004-1075, 1255, 1607-1608, 1648, 1764-1785, 2013-2014, 2056-2064, 2273-2280, which are antimicrobial agent resistance or toxin gene sequences found to be suitable for the detection and identification of microbial species.

To complement the following repertoires, another one comprising *hexA* nucleic acids and derived sequences have been construed through amplification of nucleic acids of any streptococcal species with any combination of primers SEO ID NOs.: 1179, 1181, 1182 and 1184 to 1191. From this particular repertoire, primers and/or probes for detecting *Streptococcus pneumoniae* have been designed and obtained. Particularly, a nucleic acid sequence of at least 12 nucleotides capable of hybridizing with *Streptococcus pneumoniae* and with any one of SEQ ID NOs.: 1184 to 1187 or with SEQ ID NOs.: 1179, 1180, 1181 or 1182 are provided.

The remarkable sequence diversity of nucleic acids that encode proteins also provides diversity of peptide sequences which constitute another repertoire that is also within the scope of this invention. From the protein and nucleic acid sequence repertoires is derived a use therefrom for the design of a therapeutic agent effective against a target microorganism, for example, an antibiotic, a vaccine or a genic therapeutic agent.

Due to the constant evolution in the diagnostic methods, here is finally provided a method for the identification of a microorganism in a test sample, comprising the steps of:

a) obtaining a nucleic acid sequence from a *tuf*, *fus*, *atpD*, and/or *recA* genes of said microorganisms, and

b) comparing said nucleic acid sequence with the nucleic acid sequences of a bank as defined in claim 5, said repertory comprising a nucleic acid sequence obtained from the nucleic acids of said microorganism, whereby said microorganism is identify when there is a match between the sequences.

In this method, any way by which the specified given sequence is obtained is contemplated, and this sequence is simply compared to the sequences of a bank or a repertory. If the comparison results in a match, e.g. if bank comprises the nucleic acid sequence of interest, the identification of the microorganism is provided.

DETAILED DESCRIPTION OF THE INVENTION**HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE SPECIES-SPECIFIC, GENUS-SPECIFIC, FAMILY-SPECIFIC, GROUP-SPECIFIC AND UNIVERSAL NUCLEIC ACID PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY ALGAL, ARCHAEAL, BACTERIAL, FUNGAL AND PARASITICAL MICROORGANISMS FROM CLINICAL SPECIMENS FOR DIAGNOSIS**

The present inventors reasoned that comparing the published *Haemophilus influenzae* and *Mycoplasma genitalium* genomes and searching for conserved genes could provide targets to develop useful diagnostic primers and probes. This sequence comparison is highly informative as these two bacteria are distantly related and most genes present in the minimal genome of *M. genitalium* are likely to be present in every bacterium. Therefore genes conserved between these two bacteria are likely to be conserved in all other bacteria.

Following the genomic comparison, it was found that several protein-coding genes were conserved in evolution. Highly conserved proteins included the translation elongation factors G (EF-G) and Tu (EF-Tu) and the β subunit of F₀F₁ type ATP-synthase, and to a lesser extent, the RecA recombinase. These four proteins coding genes were selected amongst the 20 most conserved genes on the basis that they all possess at least two highly conserved regions suitable for the design of universal amplification and sequencing primers. Moreover, within the fragment amplified by these primers, highly conserved and more variable regions are also present hence suggesting it might be possible to rapidly obtain sequence information from various microbial species to design universal as well as species-, genus-, family-, or group-specific primers and probes of potential use for the detection and identification and/or quantification of microorganisms.

Translation elongation factors are members of a family of GTP-binding proteins which intervene in the interactions of tRNA molecules with the ribosome machinery during essential steps of protein synthesis. The role of elongation factor Tu is to facilitate the binding of aminoacylated tRNA molecules to the A site of the ribosome. The eukaryotic, archaeal (archaeobacterial) and algal homolog of EF-Tu is called elongation factor 1 alpha (EF-1 α). All protein synthesis factors originated from a common ancestor via gene duplications and fusions (Cousineau *et al.*, 1997, J. Mol. Evol. **45**:661-670). In particular, elongation factor G (EF-G), although having a functional role in promoting the translocation of aminoacyl-tRNA molecules from the A site to the P site of the ribosome, shares sequence homologies with EF-Tu and is thought to have arisen from the duplication and fusion of an ancestor of the EF-Tu gene.

In addition, EF-Tu is known to be the target for antibiotics belonging to the elfamycin's group as well as to other structural classes (Anborgh and Parmeggiani, 1991, EMBO J. **10**:779-784; Luiten *et al.*, 1992, European patent application serial No. EP 0 466 251 A1). EF-G for its part, is the target of the antibiotic fusidic acid. In addition to its crucial activities in translation, EF-Tu has chaperone-like functions in protein folding, protection against heat denaturation of proteins and interactions with unfolded proteins (Caldas *et al.*, 1998, J. Biol. Chem **273**:11478-11482). Interestingly, a form of the EF-Tu protein has been identified as a dominant component of the periplasm of *Neisseria gonorrhoeae* (Porcella *et al.*, 1996, Microbiology **142**:2481-2489), hence suggesting that at least in some bacterial species, EF-Tu might be an antigen with vaccine potential.

F₀F₁ type ATP-synthase belongs to a superfamily of proton-translocating ATPases divided in three major families: P, V and F (Nelson and Taiz, 1989, TIBS **14**:113-116). P-ATPases (or E₁-E₂ type) operate via a phosphorylated intermediate and are not evolutionarily related to the other two families. V-ATPases (or V₀V₁ type) are present on the vacuolar and other endomembranes of eukaryotes, on the plasma membrane of archaea (archaeobacteria) and algae, and also on the plasma membrane of some eubacteria especially species belonging to the order

Spirochaetales as well as to the *Chlamydiaceae* and *Deinococcaceae* families. F-ATPases (or F₀F₁ type) are found on the plasma membrane of most eubacteria, on the inner membrane of mitochondria and on the thylakoid membrane of chloroplasts. They function mainly in ATP synthesis. They are large multimeric enzymes sharing numerous structural and functional features with the V-ATPases. F and V-type ATPases have diverged from a common ancestor in an event preceding the appearance of eukaryotes. The β subunit of the F-ATPases is the catalytic subunit and it possesses low but significant sequence homologies with the catalytic A subunit of V-ATPases.

The translation elongation factors EF-Tu, EF-G and EF-1 α , and the catalytic subunit of F or V-types ATP-synthase, are highly conserved proteins sometimes used for phylogenetic analysis and their genes are also known to be highly conserved (Iwabe *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:9355-9359, Gogarten *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:6661-6665, Ludwig *et al.*, 1993, Antonie van Leeuwenhoek **64**:285-305). A recent BLAST (Altschul *et al.*, 1997, J. Mol. Biol. **215**:403-410) search performed by the present inventors on the GenBank, European Molecular Biology Laboratory (EMBL), DNA Database of Japan (DDBJ) and specific genome project databases indicated that throughout bacteria, the EF-Tu and the β subunit of F₀F₁ type ATP-synthase genes may be more conserved than other genes that are well conserved between *H. influenzae* and *M. genitalium*.

The RecA recombinase is a multifunctional protein encoded by the *recA* gene. It plays a central role in homologous recombination, it is critical for the repair of DNA damage and it is involved in the regulation of the SOS system by promoting the proteolytic digestion of the LexA repressor. It is highly conserved in bacteria and could serve as a useful genetic marker to reconstruct bacterial phylogeny (Miller and Kokjohn, 1990, Annu. Rev. Microbiol. **44**:365-394). Although RecA possesses some highly conserved sequence segments that we used to design universal primers aimed at sequencing the *recA* fragments, it is clearly not as well conserved EF-G, EF-Tu and β subunit of F₀F₁ type ATP-synthase.

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Hence, RecA may not be optimal for universal detection of bacteria with high sensitivity but it was chosen because preliminary data indicated that EF-G, EF-Tu and β subunit of F₀F₁ type ATP-synthase may sometimes be too closely related to find specific primer pairs that could discriminate between certain very closely related species and genera. While RecA, EF-G, EF-Tu and β subunit of F₀F₁ type ATP-synthase genes, possesses highly conserved regions suitable for the design of universal sequencing primers, the less conserved region between primers should be divergent enough to allow species-specific and genus-specific primers in those cases.

Thus, as targets to design primers and probes for the genetic detection of microorganisms, the present inventors have focused on the genes encoding these four proteins: *tuf*, the gene for elongation factor Tu (EF-Tu); *fus*, the gene for the elongation factor G (EF-G); *atpD*, the gene for β subunit of F₀F₁ type ATP-synthase; and *recA*, the gene encoding the RecA recombinase. In several bacterial genomes *tuf* is often found in two highly similar duplicated copies named *tufA* and *tufB* (Filer and Furano, 1981, J. Bacteriol. 148:1006-1011, Sela *et al.*, 1989, J. Bacteriol. 171:581-584). In some particular cases, more divergent copies of the *tuf* genes can exist in some bacterial species such as some actinomycetes (Luiten *et al.* European patent application publication No. EP 0 446 251 A1; Vijgenboom *et al.*, 1994, Microbiology 140:983-998) and, as revealed as part of this invention, in several enterococcal species. In several bacterial species, *tuf* is organized in an operon with its homolog gene for the elongation factor G (EF-G) encoded by the *fusA* gene (Figure 3). This operon is often named the *str* operon. The *tuf*, *fus*, *atpD* and *recA* genes were chosen as they are well conserved in evolution and have highly conserved stretches as well as more variable segments. Moreover, these four genes have eukaryotic orthologs which are described in the present invention as targets to identify fungi and parasites. The eukaryotic homolog of elongation factor Tu is called elongation factor 1-alpha (EF-1 α) (gene name: *tef*, *tef1*, *ef1*, *ef-1* or *EF-1*). In fungi, the gene for EF-1 α occurs sometimes in two or more highly

similar duplicated copies (often named *tef1*, *tef2*, *tef3*...). In addition, eukaryotes have a copy of elongation factor Tu which is originating from their organelle genome ancestry (gene name: *tuf1*, *tufM* or *tufA*). For the purpose of the current invention, the genes for these four functionally and evolutionarily linked elongation factors (bacterial EF-Tu and EF-G, eukaryotic EF-1 α , and organellar EF-Tu) will hereafter be designated as «*tuf* nucleic acids and/or sequences». The eukaryotic (mitochondrial) F₀F₁ type ATP-synthase beta subunit gene is named *atp2* in yeast. For the purpose of the current invention, the genes of catalytic subunit of either F or V-type ATP-synthase will hereafter be designated as «*atpD* nucleic acids and/or sequences». The eukaryotic homologs of RecA are distributed in two families, typified by the Rad51 and Dmc1 proteins. Archaeal homologs of RecA are called RadA. For the purpose of the current invention, the genes corresponding to the latter proteins will hereafter be designated as «*recA* nucleic acids and/or sequences».

In the description of this invention, the terms «nucleic acids» and «sequences» might be used interchangeably. However, «nucleic acids» are chemical entities while «sequences» are the pieces of information derived from (inherent to) these «nucleic acids». Both nucleic acids and sequences are equivalently valuable sources of information for the matter pertaining to this invention.

Analysis of multiple sequence alignments of *tuf* and *atpD* sequences permitted the design of oligonucleotide primers (and probes) capable of amplifying (or hybridizing to) segments of *tuf* (and/or *fus*) and *atpD* genes from a wide variety of bacterial species (see Examples 1 to 4, 24 and 26, and Table 7). Sequencing and amplification primer pairs for *tuf* nucleic acids and/or sequences are listed in Annex I and hybridization probes are listed in Annexes III and XLVII. Sequencing and amplification primer pairs for *atpD* nucleic acids and/or sequences are listed in Annex II. Analysis of the main subdivisions of *tuf* and *atpD* sequences (see Figures 1 and 2) permitted to design sequencing primers amplifying specifically each of these subdivisions. It should be noted that these sequencing primers could also be used as universal primers. However, since some of these sequencing primers

include several variable sequence (degenerated) positions, their sensitivity could be lower than that of universal primers developed for diagnostic purposes. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

Similarly, analysis of multiple sequence alignments of *recA* sequences present in the public databases permitted the design of oligonucleotide primers capable of amplifying segments of *recA* genes from a wide variety of bacterial species. Sequencing and amplification primer pairs for *recA* sequences are listed in Annex XXI. The main subdivisions of *recA* nucleic acids and/or sequences comprise *recA*, *radA*, *rad51* and *dmc1*. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

The present inventor's strategy is to get as much sequence data information from the four conserved genes (*tuf*, *fus*, *atpD* and *recA*). This ensemble of sequence data forming a repertory (with subrepertories corresponding to each target gene and their main sequence subdivisions) and then using the sequence information of the sequence repertory (or subrepertories) to design primer pairs that could permit either universal detection of algae or archaea or bacteria or fungi or parasites, detection of a family or group of microorganism (e.g. *Enterobacteriaceae*), detection of a genus (e.g. *Streptococcus*) or finally a specific species (e.g. *Staphylococcus aureus*). It should be noted that for the purpose of the present invention a group of microorganisms is defined depending on the needs of the particular diagnostic test. It does not need to respect a particular taxonomical grouping or phylum. See Example 12 where primers were designed to amplify a group a bacteria consisting of the 17 major bacterial species encountered as contaminants of platelet concentrates. Also remark that in that Example, the primers are not only able to sensitively and rapidly detect at least the 17 important bacterial species, but could also detect other species as well, as shown in Table 14. In these circumstances the primers shown in Example 12 are considered universal for platelet-contaminating bacteria. To develop an assay specific for the latter, one or more primers or probes specific to each species could be designed. Another

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example of primers and/or probes for group detection is given by the *Pseudomonas* group primers. These primers were designed based upon alignment of *tuf* sequences from real *Pseudomonas* species as well as from former *Pseudomonas* species such as *Stenotrophomonas maltophilia*. The resulting primers are able to amplify all *Pseudomonas* species tested as well as several species belonging to different genera, hence as being specific for a group including *Pseudomonas* and other species, we defined that group as Pseudomonads, as several members were former *Pseudomonas*.

For certain applications, it may be possible to develop a universal, group, family or genus-specific reaction and to proceed to species identification using sequence information within the amplicon to design species-specific internal probes or primers, or alternatively, to proceed directly by sequencing the amplicon. The various strategies will be discussed further below.

The ensembles formed by public and proprietary *tuf*, *atpD* and *recA* nucleic acids and/or sequences are used in a novel fashion so they constitute three databases containing useful information for the identification of microorganisms.

Sequence repertoires of other gene targets were also built to solve some specific identification problems especially for microbial species genetically very similar to each other such as *E. coli* and *Shigella* (see Example 23). Based on *tuf*, *atpD* and *recA* sequences, *Streptococcus pneumoniae* is very difficult to differentiate from the closely related species *S. oralis* and *S. mitis*. Therefore, we elected to build a sequence repertoire from *hexA* sequences (Example 19), a gene much more variable than our highly conserved *tuf*, *atpD* and *recA* nucleic acids and/or sequences.

For the detection of mutations associated with antibiotic resistance genes, we also built repertoires to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. This was done for *pbp1a*, *pbp2b* and *pbp2x* genes of penicillin-resistant and sensitive *Streptococcus pneumoniae* (Example 18) and also for *gyrA* and *parC* gene fragments of various bacterial species for which quinolone resistance is important to monitor.

Oligonucleotide primers and probes design and synthesis

The *tuf*, *fus*, *atpD* and *recA* DNA fragments sequenced by us and/or selected from public databases (GenBank and EMBL) were used to design oligonucleotides primers and probes for diagnostic purposes. Multiple sequence alignments were made using subsets of the *tuf* or *atpD* or *recA* sequences repertory. Subsets were chosen to encompass as much as possible of the targetted microorganism(s) DNA sequence data and also include sequence data from phylogenetically related microorganisms from which the targetted microorganism(s) should be distinguished. Regions suitable for primers and probes should be conserved for the targetted microorganism(s) and divergent for the microorganisms from which the targetted microorganism(s) should be distinguished. The large amount of *tuf* or *atpD* or *recA* sequences data in our repertory permits to reduce trial and errors in obtaining specific and ubiquitous primers and probes. We also relied on the corresponding peptide sequences of *tuf*, *fus*, *atpD* and *recA* nucleic acids and/or sequences to facilitate the identification of regions suitable for primers and probes design. As part of the design rules, all oligonucleotides (probes for hybridization and primers for DNA amplification by PCR) were evaluated for their suitability for hybridization or PCR amplification by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo™ 5.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Oligonucleotide probes and amplification primers were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division).

The oligonucleotide sequence of primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases

A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of algae or archaea or bacteria or fungi or parasites, (ii) the species-specific detection and identification of any microorganism, including but not limited to: *Abiotrophia adiacens*, *Bacteroides fragilis*, *Bordetella pertussis*, *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitanae*, *Candida parapsilosis*, *Candida tropicalis*, *Candida zeylanoides*, *Campylobacter jejuni* and *C. coli*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Cryptococcus neoformans*, *Cryptosporidium parvum*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Escherichia coli*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Trypanosoma brucei*, *Trypanosoma cruzi*, (iii) the genus-specific detection of *Bordetella* species, *Candida* species, *Clostridium* species, *Corynebacterium* species, *Cryptococcus* species, *Entamoeba* species, *Enterococcus* species, *Gemella* species, *Giardia* species, *Legionella* species, *Leishmania* species, *Staphylococcus* species, *Streptococcus* species, *Trypanosoma* species, (iv) the family-specific detection of *Enterobacteriaceae* family members, *Mycobacteriaceae* family members, *Trypanosomatidae* family members, (v) the detection of *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus*, *Gemella* and *Abiotrophia adiacens* group, *Pseudomonads* extended group, *Platelet-contaminating bacteria* group, (vi) the detection of clinically important antimicrobial agents resistance genes listed in Table 5, (vii) the detection of clinically important toxin genes listed in Table 6.

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Variants for a given target microbial gene are naturally occurring and are attributable to sequence variation within that gene during evolution (Watson *et al.*, 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, CA; Lewin, 1989, Genes IV, John Wiley & Sons, New York, NY). For example, different strains of the same microbial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant algal, archaeal, bacterial, fungal or parasitical DNA nucleic acids and/or sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target nucleic acids and/or sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant microbial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

Sequencing of *tuf* nucleic acids and/or sequences from a variety of archaeal, bacterial, fungal and parasitical species

The nucleotide sequence of a portion of *tuf* nucleic acids and/or sequences was determined for a variety of archaeal, bacterial, fungal and parasitical species. The amplification primers (SEQ ID NOs. 664 and 697), which amplify a *tuf* gene portion of approximately 890 bp, were used along with newly designed sequencing primer pairs (See Annex I for the sequencing primers for *tuf* nucleic acids and/or

sequences). Most primer pairs can amplify different copies of *tuf* genes (*tufA* and *tufB*). This is not surprising since it is known that for several bacterial species these two genes are nearly identical. For example, the entire *tufA* and *tufB* genes from *E. coli* differ at only 13 nucleotide positions (Neidhardt *et al.*, 1996, *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed., American Society for Microbiology Press, Washington, D.C.). Similarly, some fungi are known to have two nearly identical copies of *tuf* nucleic acids and/or sequences (EF-1 α). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of *tuf* nucleic acids and/or sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The *tuf* sequencing primers even sometimes amplified highly divergent copies of *tuf* genes (*tufC*) as illustrated in the case of some enterococcal species (SEQ ID NOs.: 73, 75, 76, 614 to 618, 621 and 987 to 989). To prove this, we have determined the enterococcal *tuf* nucleic acids and/or sequences from PCR amplicons cloned into a plasmid vector. Using the sequence data from the cloned amplicons, we designed new sequencing primers specific to the divergent (*tufC*) copy of enterococci (SEQ ID NOs.: 658-659 and 661) and then sequenced directly the *tufC* amplicons. The amplification primers (SEQ ID NOs.: 543, 556, 557, 643-645, 660, 664, 694, 696 and 697) could be used to amplify the *tuf* nucleic acids and/or sequences from any bacterial species. The amplification primers (SEQ ID NOs.: 558, 559, 560, 653, 654, 655, 813, 815, 1974-1984, 1999-2003) could be used to amplify the *tuf* (EF-1 α) genes from any fungal and/or parasitical species. The amplification primers SEQ ID NOs. 1221-1228 could be used to amplify bacterial *tuf* nucleic acids and/or sequences of the EF-G subdivision (*fusA*) (Figure 3). The amplification primers SEQ ID NOs. 1224, and 1227-1229 could be used to amplify bacterial *tuf* nucleic acids and/or sequences comprising the end of EF-G (*fusA*) and the beginning of EF-Tu (*tuf*), including the intergenic region, as shown in Figure 3. Most *tuf* fragments to be sequenced were amplified using the following amplification protocol: One μ l of cell suspension (or of purified genomic DNA

0.1-100 ng/ μ l) was transferred directly to 19 μ l of a PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 1 μ M of each of the 2 primers, 200 μ M of each of the four dNTPs, 0.5 unit of *Taq* DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 94-96 °C followed by 30-45 cycles of 1 min at 95 °C for the denaturation step, 1 min at 50-55 °C for the annealing step and 1 min at 72 °C for the extension step. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The amplicons were then visualized by staining with methylene blue (Flores *et al.*, 1992, Biotechniques, 13:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, CA). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the *tuf* genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 377) with their Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The sequencing reactions were performed by using the same amplification primers and 10 ng/100 bp of the gel-purified amplicon per reaction. For the sequencing of long amplicons such as those of eukaryotic *tuf* (EF-1 α) nucleic acids and/or sequences, we designed internal sequencing primers (SEQ ID NOs.: 654, 655 and 813) to be able to obtain sequence data on both strands for most of the fragment length. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artefacts, we have sequenced two preparations of the gel-purified *tuf* amplification product originating from two independent PCR amplifications. For most target microbial species, the sequences determined for both amplicon preparations were identical. In case of discrepancies, amplicons from a third independent PCR amplification

were sequenced. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The *tuf* nucleic acids and/or sequences determined using the above strategy are described in the Sequence Listing. Table 7 gives the originating microbial species and the source for each *tuf* sequence in the Sequence Listing.

The alignment of the *tuf* sequences determined by us or selected from databases revealed clearly that the length of the sequenced portion of the *tuf* genes is variable. There may be insertions or deletions of several amino acids. In addition, in several fungi introns were observed. Intron nucleic acids and/or sequences are part of *tuf* nucleic acids and/or sequences and could be useful in the design of species-specific primers and probes. This explains why the size of the sequenced *tuf* amplification products was variable from one fungal species to another. Consequently, the nucleotide positions indicated on top of each of Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII do not correspond for sequences having insertions or deletions.

It should also be noted that the various *tuf* nucleic acids and/or sequences determined by us occasionally contain base ambiguities. These degenerated nucleotides correspond to sequence variations between *tufA* and *tufB* genes (or copies of the EF-G subdivision of *tuf* nucleic acids and/or sequences, or copies of EF-1 α subdivision of *tuf* nucleic acids and/or sequences for fungi and parasites) because the amplification primers amplify both *tuf* genes. These nucleotide variations were not attributable to nucleotide misincorporations by the *Taq* DNA polymerase because the sequence of both strands was identical and also because the sequences determined with both preparations of the gel-purified *tuf* amplicons obtained from two independent PCR amplifications were identical.

The selection of amplification primers from *tuf* nucleic acids and/or sequences

The *tuf* sequences determined by us or selected from public databases were used to select PCR primers for universal detection of bacteria, as well as for genus-

specific, species-specific family-specific or group-specific detection and identification. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various *tuf* sequences. For more details about the selection of PCR primers from *tuf* sequences please refer to Examples 5, 7-14, 17, 22, 24, 28, 30-31, 33, 36, and 38-40, and to Annexes VI-IX, XI-XIX and XXV.

Sequencing of *atpD* and *recA* nucleic acids and/or sequences from a variety of archaeal, bacterial, fungal and parasitical species

The method used to obtain *atpD* and *recA* nucleic acids and/or sequences is similar to that described above for *tuf* nucleic acids and/or sequences.

The selection of amplification primers from *atpD* or *recA* nucleic acids and/or sequences

The comparison of the nucleotide sequence for the *atpD* or *recA* genes from various archaeal, bacterial, fungal and parasitical species allowed the selection of PCR primers (refer to Examples 6, 13, 29, 34 and 37, and to Annexes IV, V, X, and XX).

DNA amplification

For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo™ 5.0 software to verify that they were good candidates for PCR amplification.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the microbial

genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing *et al*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

Briefly, the PCR protocols were as follows: Treated clinical specimens or standardized bacterial or fungal or parasitical suspensions (see below) or purified genomic DNA from bacteria, fungi or parasites were amplified in a 20 μ l PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM MgCl₂, 0.4 μ M of each primer, 200 μ M of each of the four dNTPs and 0.5 unit of *Taq* DNA polymerase (Promega) combined with the TaqStartTM antibody (Clontech Laboratories Inc., Palo Alto, CA). The TaqStartTM antibody, which is a neutralizing monoclonal antibody to *Taq* DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg *et al.*, 1994, Biotechniques 16:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the microbial cells and eliminate or neutralize PCR inhibitors. For amplification from bacterial or fungal or parasitical cultures or from purified genomic DNA, the samples were added directly to the PCR amplification mixture without any pre-treatment step. An internal control was derived from sequences not found in the target microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. Alternatively, an internal control derived from rRNA was also useful to monitor the efficiency of microbial lysis protocols.

PCR reactions were then subjected to thermal cycling (3 min at 94-96°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 50-65°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.). The number of cycles performed for the PCR assays varies

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according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are probably required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal or parasitical cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase amplification (SPA), rolling circle amplification technology (RCA), solid phase RCA, anchored SDA and nuclease dependent signal amplification (NDSA) (Lee *et al.*, 1997, *Nucleic Acid Amplification Technologies: Application to Disease Diagnosis*, Eaton Publishing, Boston, MA; Persing *et al.*, 1993, *Diagnostic Molecular Microbiology: Principles and Applications*, American Society for Microbiology, Washington, D.C.; Westin *et al.*, 2000, *Nat. Biotechnol.* 18:199-204). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase the sensitivity and/or the rapidity of nucleic acid-based diagnostic tests. The scope of the present invention also covers the use of any nucleic acids amplification and detection technology including real-time or post-amplification detection technologies, any amplification technology combined with detection, any hybridization nucleic acid chips or arrays technologies, any amplification chips or combination of amplification and

hybridization chips technologies. Detection and identification by any sequencing method is also under the scope of the present invention.

Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR or for DNA hybridization which are derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antimicrobial agents resistance or toxin gene sequences included in this document are also under the scope of this invention.

Detection of amplification products

Classically, detection of amplification is performed by standard ethidium bromide-stained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after or during amplification. One simple method for monitoring amplified DNA is to measure its rate of formation by measuring the increase in fluorescence of intercalating agents such as ethidium bromide or SYBR[®] Green I (Molecular Probes). If more specific detection is required, fluorescence-based technologies can monitor the appearance of a specific product during the reaction. The use of dual-labeled fluorogenic probes such as in the TaqMan[™] system (Applied Biosystems) which utilizes the 5'-3' exonuclease activity of the *Taq* polymerase is a good example (Livak K.J. *et al.* 1995, PCR Methods Appl. 4:357-362). TaqMan[™] can be performed during amplification and this "real-time" detection can be done in a single closed tube hence eliminating post-PCR sample handling and consequently preventing the risk of amplicon carryover. Several other fluorescence-based detection methods can be performed in real-time. Fluorescence resonance energy transfer (FRET) is the principle behind the use of adjacent hybridization probes (Wittwer, C.T. *et al.* 1997. BioTechniques 22:130-138), molecular beacons (Tyagi S. and Kramer F.R. 1996. Nature Biotechnology 14:303-308) and scorpions (Whitcomb *et al.* 1999. Nature

Biotechnology 17:804-807). Adjacent hybridization probes are designed to be internal to the amplification primers. The 3' end of one probe is labelled with a donor fluorophore while the 5' end of an adjacent probe is labelled with an acceptor fluorophore. When the two probes are specifically hybridized in closed proximity (spaced by 1 to 5 nucleotides) the donor fluorophore which has been excited by an external light source emits light that is absorbed by a second acceptor that emit more fluorescence and yields a FRET signal. Molecular beacons possess a stem-and-loop structure where the loop is the probe and at the bottom of the stem a fluorescent moiety is at one end while a quenching moiety is at the other end. The beacons undergo a fluorogenic conformational change when they hybridize to their targets hence separating the fluorochrome from its quencher. The FRET principle is also used in an air thermal cycler with a built-in fluorometer (Wittwer, C.T. *et al.* 1997. *BioTechniques* 22:130-138). The amplification and detection are extremely rapid as reactions are performed in capillaries: it takes only 18 min to complete 45 cycles. Those techniques are suitable especially in the case where few pathogens are searched for. Boehringer-Roche Inc. sells the LightCycler™, and Cepheid makes the SmartCycler. These two apparatus are capable of rapid cycle PCR combined with fluorescent SYBR® Green I or FRET detection. We recently demonstrated in our laboratory, real-time detection of 10 CFU in less than 40 minutes using adjacent hybridization probes on the LightCycler™. Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated.

Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any sequence from our repertory and designed to specifically hybridize to DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus or family or group detection and identification may be derived from the amplicons produced by a universal, family-, group-, genus- or species-specific amplification assay(s). The oligonucleotide

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probes may be labeled with biotin or with digoxigenin or with any other reporter molecule (for more details see below the section on hybrid capture). Hybridization on a solid support is amendable to miniaturization.

At present the oligonucleotide nucleic acid microarray technology is appealing. Currently, available low to medium density arrays (Heller *et al.*, An integrated microelectronics hybridization system for genomic research and diagnostic applications. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.) could specifically capture fluorescent-labelled amplicons. Detection methods for hybridization are not limited to fluorescence; potentiometry, colorimetry and plasmon resonance are some examples of alternative detection methods. In addition to detection by hybridization, nucleic acid microarrays could be used to perform rapid sequencing by hybridization. Mass spectrometry could also be applicable for rapid identification of the amplicon or even for sequencing of the amplification products (Chiu and Cantor, 1999, Clinical Chemistry **45**:1578; Berkenkamp *et al.*, 1998, Science **281**:260).

For the future of our assay format, we also consider the major challenge of molecular diagnostics tools, *i.e.*: integration of the major steps including sample preparation, genetic amplification, detection, data analysis and presentation (Anderson *et al.*, Advances in integrated genetic analysis. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.).

To ensure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York). The concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and MgCl₂ are 0.1-1.5 μ M and

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1.0-10.0 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples.

Hybrid capture and chemiluminescence detection of amplification products

Hybridization and detection of amplicons by chemiluminescence were adapted from Nikiforov *et al.* (1994, PCR Methods and Applications 3:285-291 and 1995, Anal. Biochem. 227:201-209) and from the DIG™ system protocol of Boehringer Mannheim. Briefly, 50 µl of a 25 picomoles solution of capture probe diluted in EDC {1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride} are immobilized in each well of 96-wells plates (Microlite™ 2, Dynex) by incubation overnight at room temperature. The next day, the plates are incubated with a solution of 1% BSA diluted into TNTw (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.05% Tween™ 20) for 1 hour at 37 °C. The plates are then washed on a Wellwash Ascent™ (Labsystems) with TNTw followed by Washing Buffer (100 mM maleic acid pH7.5; 150 mM NaCl; 0.3% Tween™ 20).

The amplicons were labelled with DIG-11-dUTP during PCR using the PCR DIG Labelling Mix from Boehringer Mannheim according to the manufacturer's instructions. Hybridization of the amplicons to the capture probes is performed in triplicate at stringent temperature (generally, probes are designed to allow hybridization at 55 °C, the stringent temperature) for 30 minutes in 1.5 M NaCl; 10 mM EDTA. It is followed by two washes in 2 X SSC; 0.1% SDS, then by four washes in 0.1X SSC; 0.1% SDS at the stringent temperature (55 °C). Detection with 1,2 dioxetane chemiluminescent alkaline phosphatase substrates like CSPD® (Tropix Inc.) is performed according to the manufacturer's instructions but with shorter incubations times and a different antibody concentration. The plates are

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agitated at each step, the blocking incubation is performed for only 5 minutes, the anti-DIG-AP1 is used at a 1:1000 dilution, the incubation with antibody lasts 15 minutes, the plates are washed twice for only 5 minutes. Finally, after a 2 minutes incubation into the detection buffer, the plates are incubated 5 minutes with CSPD® at room temperature followed by a 10 minutes incubation at 37 °C without agitation. Luminous signal detection is performed on a Dynex Microtiter Plate Luminometer using RLU (Relative Light Units).

Specificity, ubiquity and sensitivity tests for oligonucleotide primers and probes

The specificity of oligonucleotide primers and probes was tested by amplification of DNA or by hybridization with bacterial or fungal or parasitical species selected from a panel comprising closely related species and species sharing the same anatomo-pathological site (see Annexes and Examples). All of the bacterial, fungal and parasitical species tested were likely to be pathogens associated with infections or potential contaminants which can be isolated from clinical specimens. Each target DNA could be released from microbial cells using standard chemical and/or physical treatments to lyse the cells (Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) or alternatively, genomic DNA purified with the GNOMETM DNA kit (Bio101, Vista, CA) was used. Subsequently, the DNA was subjected to amplification with the primer pairs. Specific primers or probes amplified only the target microbial species, genus, family or group.

Oligonucleotides primers found to amplify specifically the target species, genus, family or group were subsequently tested for their ubiquity by amplification (i.e. ubiquitous primers amplified efficiently most or all isolates of the target species or genus or family or group). Finally, the sensitivity of the primers or probes was determined by using 10-fold or 2-fold dilutions of purified genomic DNA from the targeted microorganism. For most assays, sensitivity levels in the

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range of 1-100 copies were obtained. The specificity, ubiquity and sensitivity of the PCR assays using the selected amplification primer pairs were tested either directly from cultures of microbial species or from purified microbial genomic DNA.

Probes were tested in hybrid capture assays as described above. An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus or family or group from which it was selected. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes detected efficiently most or all isolates of the target species or genus or family or group) by hybridization to microbial DNAs from different clinical isolates of the species or genus or family or group of interest including ATCC reference strains. Similarly, oligonucleotide primers and probes could be derived from antimicrobial agents resistance or toxin genes which are objects of the present invention.

Reference strains

The reference strains used to build proprietary *tuf*, *atpD* and *recA* sequence data subrepertories, as well as to test the amplification and hybridization assays were obtained from (i) the American Type Culture Collection (ATCC), (ii) the Laboratoire de santé publique du Québec (LSPQ), (iii) the Centers for Disease Control and Prevention (CDC), (iv) the National Culture Type Collection (NCTC) and (v) several other reference laboratories throughout the world. The identity of our reference strains was confirmed by phenotypic testing and reconfirmed by analysis of *tuf*, *atpD* and *recA* sequences (see Example 13).

Antimicrobial agents resistance genes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of

microbial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal algal, archaeal, bacterial, fungal or parasitical detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians also need timely information about the ability of the microbial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly microbial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antimicrobial agents resistance genes (i.e. DNA-based tests for the specific detection of antimicrobial agents resistance genes). Since the sequence from the most important and common antimicrobial agents resistance genes are available from public databases, our strategy is to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the antimicrobial agents resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 5; descriptions of the designed amplification primers and internal probes are given in Annexes XXXIV-XXXVII, XXXIX, XLV, and L-LI. Our approach is unique because the antimicrobial agents resistance genes detection and the microbial detection and identification can be performed simultaneously, or independently, or sequentially in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Toxin genes

Toxin identification is often very important to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a

specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians sometimes need timely information about the ability of certain bacterial pathogens to produce toxins. Since the sequence from the most important and common bacterial toxin genes are available from public databases, our strategy is to use the sequence from a portion or from the entire toxin gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the bacterial toxin genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 6; descriptions of the designed amplification primers and internal probes are given in Annexes XXII, XXXII and XXXIII. Our approach is unique because the toxin genes detection and the bacterial detection and identification can be performed simultaneously, or independently, or sequentially, in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Universal bacterial detection

In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture. Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screening out the numerous negative specimens is thus useful as it reduces costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the *tuf*, *atpD* and *recA* nucleic acids and/or sequences. The universal primers selection was based on a multiple sequence alignment constructed with sequences from our repertory.

All computer analysis of amino acid and nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for

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the universal amplification of bacteria were selected with the help of the Oligo™ program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of base ambiguities in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

The amplification conditions with the universal primers are very similar to those used for the species- and genus-specific amplification assays except that the annealing temperature is slightly lower. The original universal PCR assay described in our assigned WO98/20157 (SEQ ID NOs. 23-24 of the latter application) was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species as well as genomic DNA from *Leishmania donovani*, *Saccharomyces cerevisiae* and human lymphocytes. None of the above eukaryotic DNA preparations could be amplified by the universal assay, thereby suggesting that this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Table 4. We found that at least 104 of these strains could be amplified. However, the assay could be improved since bacterial species which could not be amplified with the original *tuf* nucleic acids and/or sequences-based assay included species belonging to the following genera: *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). Sequencing of the *tuf* genes from these bacterial species and others has been performed in the scope of the present invention in order to improve the universal assay. This

sequencing data has been used to select new universal primers which may be more ubiquitous and more sensitive. Also, we improved our primer and probes design strategy by taking into consideration the phylogeny observed in analysing our repertoire of *tuf*, *atpD* and *recA* sequences. Data from each of the 3 main subrepertoires (*tuf*, *atpD* and *recA*) was subjected to a basic phylogenetic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group, inc.). This analysis indicated the main branches or phyla reflecting the relationships between sequences. Instead of trying to design primers or probes able to hybridize to all phyla, we designed primers or probes able to hybridize to the main phyla while trying to use the largest phylum possible. This strategy should allow less degenerated primers hence improving sensitivity and by combining primers in a mutiplex assay, improve ubiquity. Universal primers SEQ ID NOs. 643-645 based on *tuf* sequences have been designed to amplify most pathogenic bacteria except *Actinomycetaceae*, *Clostridiaceae* and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (pathogenic bacteria of this phylum include mostly *Bacteroides*, *Porphyromonas* and *Prevotella* species). Primers to fill these gaps have been designed for *Actinomycetaceae* (SEQ ID NOs. 646-648), *Clostridiaceae* (SEQ ID NOs. 796-797, 808-811), and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (SEQ ID NOs. 649-651), also derived from *tuf* nucleic acids and/or sequences. These primers sets could be used alone or in conjunction to render the universal assay more ubiquitous.

Universal primers derived from *atpD* sequences include SEQ ID NOs. 562-565. Combination of these primers does not amplify human DNA but should amplify almost all pathogenic bacterial species except proteobacteria belonging to the epsilon subdivision (*Campylobacter* and *Helicobacter*), the bacteria from the *Cytophaga*, *Flexibacter* and *Bacteroides* group and some actinomycetes and corynebacteria. By analysing *atpD* sequences from the latter species, primers and probes to specifically fill these gaps could be designed and used in conjunction with primers SEQ ID NOs. 562-565, also derived from *atpD* nucleic acids and/or sequences.

In addition, universality of the assay could be expanded by mixing *atpD* sequences-derived primers with *tuf* sequences-derived primers. Ultimately, even *recA* sequences-derived primers could be added to fill some gaps in the universal assay.

It is important to note that the 95 bacterial species selected to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

Amino acid sequences derived from *tuf*, *atpD* and *recA* nucleic acids and/or sequences

The amino acid sequences translated from the repertory of *tuf*, *atpD* and *recA* nucleic acids and/or sequences are also an object of the present invention. The amino acid sequence data will be particularly useful for homology modeling of three-dimensional (3D) structure of the elongation factor Tu, elongation factor G, elongation factor 1 α , ATPase subunit beta and RecA recombinase. For all these proteins, at least one structure model has been published using X-ray diffraction data from crystals. Based on those structural informations it is possible to use computer software to build 3D model structures for any other protein having peptide sequence homologies with the known structure (Greer, 1991, Methods in Enzymology, **202**:239-252; Taylor, 1994, Trends Biotechnol., **12**(5):154-158; Sali, 1995, Curr. Opin. Biotechnol. **6**:437-451; Sanchez and Sali, 1997, Curr. Opin. Struct. Biol. **7**:206-214; Fischer and Eisenberg, 1999, Curr. Opin. Struct. Biol. **9**:208-211; Guex *et al.*, 1999, Trends Biochem. Sci. **24**: 364-367). Model structures of target proteins are used for the design or to predict the behavior of ligands and inhibitors such as antibiotics. Since EF-Tu and EF-G are already known as antibiotic targets (see above) and since the beta subunit of ATPase and RecA recombinase are essential to the survival of the microbial cells in natural

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conditions of infection, all four proteins could be considered antibiotic targets. Sequence data, especially the new data generated by us could be very useful to assist the creation of new antibiotic molecules with desired spectrum of activity. In addition, model structures could be used to improve protein function for commercial purposes such as improving antibiotic production by microbial strains or increasing biomass.

The following detailed embodiments and appended drawings are provided as illustrative examples of his invention, with no intention to limit the scope thereof.

DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 illustrate the principal subdivisions of the *tuf* and *atpD* sequences repertoires, respectively. For the design of primers and probes, depending on the needs, one may want to use the complete data set illustrated on the top of the pyramid or use only a subset illustrated by the different branching points. Smaller subdivisions, representing groups, families, genus and species, could even be made to extend to the bottom of the pyramid. Because the *tuf* and *atpD* sequences are highly conserved and evolved with each species, the design of primers and probes does not need to include all the sequences within the database or its subdivisions. As illustrated in Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII, depending on the use, sequences from a limited number of species can be carefully selected to represent: i) only the main phylogenetic branches from which the intended probes and primers need to be differentiating, and ii) only the species for which they need to be matching. However, for ubiquity purposes, and especially for primers and probes identifying large groups of species (genus, family, group or universal, or sequencing primers), the more data is included into the sequence analysis, the better the probes and primers will be suitable for each particular intended use. Similarly, for specificity purposes, a larger data set (or repertoire) ensures optimal primers and probes design by reducing the chance of employing nonspecific oligonucleotides.

Figure 3 illustrates the approach used to design specific amplification primers from *fusA* as well as from the region between the end of *fusA* and the beginning of *tuf* in the streptomycin (*str*) operon (referred to as the *fusA-tuf* intergenic spacer in Table 7).

Figures 4 to 6 are illustrations to Example 42, whereas Figures 7 to 10 illustrate Example 43. Figures 11 and 12 illustrate Example 44.

FIGURE LEGENDS

Figure 3. Schematic organization of universal amplification primers (SEQ ID NOs. 1221-1229) in the *str* operon. Amplicon sizes are given in bases pairs. Drawing not to scale, as the *fusA-tuf* intergenic spacer size varies depending on the bacterial species. Indicated amplicon lengths are for *E. coli*.

Figure 4. Abridged multiple amino acid sequence alignment of the partial *tuf* gene products from selected species illustrated using the program Alscript. Residues highly conserved in bacteria are boxed in grey and gaps are represented with dots. Residues in reverse print are unique to the enterococcal *tufB* as well as to streptococcal and lactococcal *tuf* gene products. Numbering is based on *E. coli* EF-Tu and secondary structure elements of *E. coli* EF-Tu are represented by cylinders (α -helices) and arrows (β -strands).

Figure 5. Distance matrix tree of bacterial EF-Tu based on amino acid sequence homology. The tree was constructed by the neighbor-joining method. The tree was rooted using archeal and eukaryotic EF-1 α genes as the outgroup. The scale bar represents 5% changes in amino acid sequence, as determined by taking the sum of all of the horizontal lines connecting two species.

Figure 6. Southern hybridization of *Bgl*III/*Xba*I digested genomic DNAs of some enterococci (except for *E. casseliflavus* and *E. gallinarum* whose genomic DNA was digested with *Bam*HI/*Pvu*II) using the *tufA* gene fragment of *E. faecium* as probes. The sizes of hybridizing fragments are shown in kilobases. Strains tested are listed in Table 16.

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Figure 7. *Pantoea* and *Tatumella* species specific signature indel in *atpD* genes. The nucleotide positions given are for *E. coli atpD* sequence (GenBank accession no. V00267). Numbering starts from the first base of the initiation codon.

Figure 8: Trees based on sequence data from *tuf* (left side) and *atpD* (right side). The phylogenetic analysis was performed using the Neighbor-Joining method calculated using the Kimura two-parameter method. The value on each branch indicates the occurrence (%) of the branching order in 750 bootstrapped trees.

Figure 9: Phylogenetic tree of members of the family *Enterobacteriaceae* based on *tuf* (a), *atpD* (b), and 16S rDNA (c) genes. Trees were generated by neighbor-joining method calculated using the Kimura two-parameter method. The value on each branch is the percentage of bootstrap replications supporting the branch. 750 bootstrap replications were calculated.

Figure 10: Plot of *tuf* distances versus 16S rDNA distances (a), *atpD* distances versus 16S rDNA distances (b), and *atpD* distances versus *tuf* distances (c). Symbols: ○, distances between pairs of strains belonging to the same species; ●, distances between *E. coli* strains and *Shigella* strains; □, distances between pairs belonging to the same genus; ■, distances between pairs belonging to different genera; △, distances between pairs belonging to different families.

EXAMPLES AND ANNEXES

For sake of clarity, here is a list of Examples and Annexes:

Example 1: Sequencing of bacterial *atpD* (F-type and V-type) gene fragments.

Example 2: Sequencing of eukaryotic *atpD* (F-type and V-type) gene fragments.

Example 3: Sequencing of eukaryotic *tuf* (EF-1) gene fragments.

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- Example 4: Sequencing of eukaryotic *tuf* (organelle origin, M) gene fragments.
- Example 5: Specific detection and identification of *Streptococcus agalactiae* using *tuf* sequences.
- Example 6: Specific detection and identification of *Streptococcus agalactiae* using *atpD* sequences.
- Example 7: Development of a PCR assay for detection and identification of staphylococci at genus and species levels.
- Example 8: Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis*.
- Example 9: Specific detection and identification of *Entamoeba histolytica*.
- Example 10: Sensitive detection and identification of *Chlamydia trachomatis*.
- Example 11: Genus-specific detection and identification of enterococci.
- Example 12: Detection and identification of the major bacterial platelets contaminants using *tuf* sequences with a multiplex PCR test.
- Example 13: The resolving power of the *tuf* and *atpD* sequences databases is comparable to the biochemical methods for bacterial identification.
- Example 14: Detection of group B streptococci from clinical specimens.
- Example 15: Simultaneous detection and identification of *Streptococcus pyogenes* and its pyrogenic exotoxin A.
- Example 16: Real-time detection and identification of Shiga toxin-producing bacteria.
- Example 17: Development of a PCR assay for the detection and identification of staphylococci at genus and species levels and its associated *mecA* gene.
- Example 18: Sequencing of *pbp1a*, *pbp2b* and *pbp2x* genes of *Streptococcus pneumoniae*.
- Example 19: Sequencing of *hexA* genes of *Streptococcus* species.
- Example 20: Development of a multiplex PCR assay for the detection of *Streptococcus pneumoniae* and its penicillin resistance genes.

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- Example 21: Sequencing of the vancomycin resistance *vanA*, *vanC1*, *vanC2* and *vanC3* genes.
- Example 22: Development of a PCR assay for the detection and identification of enterococci at genus and species levels and its associated resistance genes *vanA* and *vanB*.
- Example 23: Development of a multiplex PCR assay for detection and identification of vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*.
- Example 24: Universal amplification involving the EF-G (*fusA*) subdivision of *tuf* sequences.
- Example 25: DNA fragment isolation from *Staphylococcus saprophyticus* by arbitrarily primed PCR.
- Example 26: Sequencing of prokaryotic *tuf* gene fragments.
- Example 27: Sequencing of procaryotic *recA* gene fragments.
- Example 28: Specific detection and identification of *Escherichia coli*/*Shigella* sp. using *tuf* sequences.
- Example 29: Specific detection and identification of *Klebsiella pneumoniae* using *atpD* sequences.
- Example 30: Specific detection and identification of *Acinetobacter baumannii* using *tuf* sequences.
- Example 31: Specific detection and identification of *Neisseria gonorrhoeae* using *tuf* sequences.
- Example 32: Sequencing of bacterial *gyrA* and *parC* gene fragments.
- Example 33: Development of a PCR assay for the specific detection and identification of *Staphylococcus aureus* and its quinolone resistance genes *gyrA* and *parC*.
- Example 34: Development of a PCR assay for the detection and identification of *Klebsiella pneumoniae* and its quinolone resistance genes *gyrA* and *parC*.

- Example 35: Development of a PCR assay for the detection and identification of *Streptococcus pneumoniae* and its quinolone resistance genes *gyrA* and *parC*.
- Example 36: Detection of extended-spectrum TEM-type β -lactamases in *Escherichia coli*.
- Example 37: Detection of extended-spectrum SHV-type β -lactamases in *Klebsiella pneumoniae*.
- Example 38: Development of a PCR assay for the detection and identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene *tetM*.
- Example 39: Development of a PCR assay for the detection and identification of *Shigella* sp. and their associated trimethoprim resistance gene *dhfr1a*.
- Example 40: Development of a PCR assay for the detection and identification of *Acinetobacter baumannii* and its associated aminoglycoside resistance gene *aph(3')-VIa*.
- Example 41: Specific detection and identification of *Bacteroides fragilis* using *atpD* (V-type) sequences.
- Example 42: Evidence for horizontal gene transfer in the evolution of the elongation factor Tu in Enterococci.
- Example 43: Elongation factor Tu (*tuf*) and the F-ATPase beta-subunit (*atpD*) as phylogenetic tools for species of the family *Enterobacteriaceae*.
- Example 44: Testing new pairs of PCR primers selected from two species-specific genomic DNA fragments which are objects of US patent 6,001,564.
- Example 45: Testing modified versions of PCR primers derived from the sequence of several primers which are objects of US patent 6,001,564.

The various Annexes show the strategies used for the selection of a variety of DNA amplification primers, nucleic acid hybridization probes and molecular beacon internal probes:

- (i) Annex I shows the amplification primers used for nucleic acid amplification from *tuf* sequences.
- (ii) Annex II shows the amplification primers used for nucleic acid amplification from *atpD* sequences.
- (iii) Annex III shows the internal hybridization probes for detection of *tuf* sequences.
- (iv) Annex IV illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the F-type.
- (v) Annex V illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the V-type.
- (vi) Annex VI illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of organelle lineage (M, the letter M is used to indicate that in most cases, the organelle is the mitochondria).
- (vii) Annex VII illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of eukaryotes (EF-1).
- (viii) Annex VIII illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *tuf* sequences.
- (ix) Annex IX illustrates the strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from *tuf* sequences.
- (x) Annex X illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *atpD* sequences.
- (xi) Annex XI illustrates the strategy for the selection from *tuf* sequences of *Candida albicans/dubliniensis*-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe.

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- (xii) Annex XII illustrates the strategy for the selection of *Staphylococcus*-specific amplification primers from *tuf* sequences.
- (xiii) Annex XIII illustrates the strategy for the selection of the *Staphylococcus*-specific hybridization probe from *tuf* sequences.
- (xiv) Annex XIV illustrates the strategy for the selection of *Staphylococcus saprophyticus*-specific and *Staphylococcus haemolyticus*-specific hybridization probes from *tuf* sequences.
- (xv) Annex XV illustrates the strategy for the selection of *Staphylococcus aureus*-specific and *Staphylococcus epidermidis*-specific hybridization probes from *tuf* sequences.
- (xvi) Annex XVI illustrates the strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from *tuf* sequences.
- (xvii) Annex XVII illustrates the strategy for the selection of the *Enterococcus*-specific amplification primers from *tuf* sequences.
- (xviii) Annex XVIII illustrates the strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from *tuf* sequences.
- (xix) Annex XIX illustrates the strategy for the selection of primers from *tuf* sequences for the identification of platelets contaminants.
- (xx) Annex XX illustrates the strategy for the selection of the universal amplification primers from *atpD* sequences.
- (xxi) Annex XXI shows the amplification primers used for nucleic acid amplification from *recA* sequences.
- (xxii) Annex XXII shows the specific and ubiquitous primers for nucleic acid amplification from *speA* sequences.
- (xxiii) Annex XXIII illustrates the first strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *speA* sequences.

- (xxiv) Annex XXIV illustrates the second strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *speA* sequences.
- (xxv) Annex XXV illustrates the strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from *tuf* sequences.
- (xxvi) Annex XXVI illustrates the strategy for the selection of *stx*₁-specific amplification primers and hybridization probe.
- (xxvii) Annex XXVII illustrates the strategy for the selection of *stx*₂-specific amplification primers and hybridization probe.
- (xxviii) Annex XXVIII illustrates the strategy for the selection of *vanA*-specific amplification primers from *van* sequences.
- (xxix) Annex XXIX illustrates the strategy for the selection of *vanB*-specific amplification primers from *van* sequences.
- (xxx) Annex XXX illustrates the strategy for the selection of *vanC*-specific amplification primers from *vanC* sequences.
- (xxxi) Annex XXXI illustrates the strategy for the selection of *Streptococcus pneumoniae*-specific amplification primers and hybridization probes from *pbplA* sequences.
- (xxxii) Annex XXXII shows the specific and ubiquitous primers for nucleic acid amplification from toxin gene sequences.
- (xxxiii) Annex XXXIII shows the molecular beacon internal hybridization probes for specific detection of toxin sequences.
- (xxxiv) Annex XXXIV shows the specific and ubiquitous primers for nucleic acid amplification from *van* sequences.
- (xxxv) Annex XXXV shows the internal hybridization probes for specific detection of *van* sequences.
- (xxxvi) Annex XXXVI shows the specific and ubiquitous primers for nucleic acid amplification from *pbp* sequences.
- (xxxvii) Annex XXXVII shows the internal hybridization probes for specific detection of *pbp* sequences.

- (xxxviii) Annex XXXVIII illustrates the strategy for the selection of *vanAB*-specific amplification primers and *vanA*- and *vanB*- specific hybridization probes from *van* sequences.
- (xxxix) Annex XXXIX shows the internal hybridization probe for specific detection of *mecA*.
- (xl) Annex XL shows the specific and ubiquitous primers for nucleic acid amplification from *hexA* sequences.
- (xli) Annex XLI shows the internal hybridization probe for specific detection of *hexA*.
- (xlii) Annex XLII illustrates the strategy for the selection of *Streptococcus pneumoniae* species-specific amplification primers and hybridization probe from *hexA* sequences.
- (xliii) Annex XLIII shows the specific and ubiquitous primers for nucleic acid amplification from *pcp* sequences.
- (xliv) Annex XLIV shows specific and ubiquitous primers for nucleic acid amplification of *S. saprophyticus* sequences of unknown coding potential.
- (xlv) Annex XLV shows the molecular beacon internal hybridization probes for specific detection of antimicrobial agents resistance gene sequences.
- (xlvi) Annex XLVI shows the molecular beacon internal hybridization probe for specific detection of *S. aureus* gene sequences of unknown coding potential.
- (xlvii) Annex XLVII shows the molecular beacon hybridization internal probe for specific detection of *tuf* sequences.
- (xlviii) Annex XLVIII shows the molecular beacon internal hybridization probes for specific detection of *ddl* and *mtl* sequences.
- (xlix) Annex XLIX shows the internal hybridization probe for specific detection of *S. aureus* sequences of unknown coding potential.
- (l) Annex L shows the amplification primers used for nucleic acid amplification from antimicrobial agents resistance genes sequences.

- (li) Annex LI shows the internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.
- (lii) Annex LII shows the molecular beacon internal hybridization probes for specific detection of *atpD* sequences.
- (liii) Annex LIII shows the internal hybridization probes for specific detection of *atpD* sequences.
- (liv) Annex LIVI shows the internal hybridization probes for specific detection of *ddl* and *mtl* sequences.

As shown in these Annexes, the selected amplification primers may contain inosines and/or base ambiguities. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degeneracies in the amplification primers allows mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York).

EXAMPLES

EXAMPLE 1:

Sequencing of bacterial *atpD* (F-type and V-type) gene fragments. As shown in Annex IV, the comparison of publicly available *atpD* (F-type) sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences (F-type) from a wide range of bacterial species. Using primers pairs SEQ ID NOs. 566 and 567, 566 and 814, 568 and 567, 570 and 567, 572 and 567, 569 and 567, 571 and 567, 700 and 567, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 242-270, 272-398, 673-

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674, 737-767, 866-867, 942-955, 1245-1254, 1256-1265, 1527, 1576, 1577, 1600-1604, 1640-1646, 1649, 1652, 1655, 1657, 1659-1660, 1671, 1844-1845, and 1849-1865.

Similarly, Annex V shows the strategy to design the PCR primers able to amplify *atpD* sequences of the V-type from a wide range of archaeal and bacterial species. Using primers SEQ ID NOs. 681-683, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 827-832, 929-931, 958 and 966. As the gene was difficult to amplify for several species, additional amplification primers were designed inside the original amplicon (SEQ ID NOs. 1203-1207) in order to obtain sequence information for these species. Other primers (SEQ ID NO. 1212, 1213, 2282-2285) were also designed to amplify regions of the *atpD* gene (V-type) in archaeobacteria.

EXAMPLE 2:

Sequencing of eukaryotic *atpD* (F-type and V-type) gene fragments. The comparison of publicly available *atpD* (F-type) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 568 and 573, 574 and 573, 574 and 708, and 566 and 567, it was possible to amplify and sequence *atpD* sequences SEQ ID NOs. 458-497, 530-538, 663, 667, 676, 678-680, 768-778, 856-862, 889-896, 941, 1638-1639, 1647, 1650-1651, 1653-1654, 1656, 1658, 1684, 1846-1848, and 2189-2192.

In the same manner, the primers described in Annex V (SEQ ID NOs. 681-683) could amplify the *atpD* (V-type) gene from various fungal and parasitical species. This strategy allowed to obtain SEQ ID NOs. 834-839, 956-957, and 959-965.

EXAMPLE 3:

Sequencing of eukaryotic *tuf* (EF-1) gene fragments. As shown in Annex VII, the comparison of publicly available *tuf* (EF-1) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 558 and 559, 813 and 559, 558 and 815, 560 and 559, 653 and 559, 558 and 655, and 654 and 559, 1999 and 2000, 2001 and 2003, 2002 and 2003, it was possible to amplify and sequence *tuf* sequences SEQ ID NOs. 399-457, 509-529, 622-624, 677, 779-790, 840-842, 865, 897-903, 1266-1287, 1561-1571 and 1685.

EXAMPLE 4:

Sequencing of eukaryotic *tuf* (organelle origin, M) gene fragments. As shown in Annex VI, the comparison of publicly available *tuf* (organelle origin, M) sequences from a variety of fungal and parasitical organelles revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences of several organelles belonging to a wide range fungal and parasitical species. Using primers pairs SEQ ID NOs. 664 and 652, 664 and 561, 911 and 914, 912 and 914, 913 and 915, 916 and 561, 664 and 917, it was possible to amplify and sequence *tuf* sequences SEQ ID NOs. 498-508, 791-792, 843-855, 904-910, 1664, 1666-1667, 1669-1670, 1673-1683, 1686-1689, 1874-1876, 1879, 1956-1960, and 2193-2199.

EXAMPLE 5:

Specific detection and identification of *Streptococcus agalactiae* using *tuf* sequences. As shown in Annex VIII, the comparison of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The strategy used to design the PCR primers was based on the analysis

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of a multiple sequence alignment of various *tuf* sequences. The multiple sequence alignment includes the *tuf* sequences of four bacterial strains from the target species as well as *tuf* sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species and genera, especially from the closely related species, thereby permitting the species-specific, ubiquitous and sensitive detection and identification of the target bacterial species.

The chosen primer pair, oligos SEQ ID NO. 549 and SEQ ID NO. 550, gives an amplification product of 252 bp. Standard PCR was carried out using 0.4 μ M of each primer, 2.5 mM MgCl₂, BSA 0.05 mM, 1X Taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStartTM antibody (Clontech Laboratories Inc., Palo Alto), 1 μ l of genomic DNA sample in a final volume of 20 μ l using a PTC-200 thermocycler (MJ Research Inc.). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μ g/ml of ethidium bromide.

Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the bacterial species listed in Table 8. Efficient amplification was observed only for the 5 *S. agalactiae* strains listed. Of the other bacterial species, including 32 species representative of the vaginal flora and 27 other streptococcal species, only *S. acidominimus* yielded amplification. The signal with 0.1 ng of *S. acidominimus* genomic DNA was weak and the detection limit for this species was 10 pg (corresponding to more than 4000 genome copies) while the detection limit for *S. agalactiae* was 2.5 fg (corresponding to one genome copy) of genomic DNA.

To increase the specificity of the assay, internal probes were designed for FRET (Fluorescence Resonance Energy Transfer) detection using the LightCycler™ (Idaho Technology). As illustrated in Annex IX, a multiple sequence alignment of streptococcal *tuf* sequence fragments corresponding to the 252 bp region amplified by primers SEQ ID NO. 549 and SEQ ID NO. 550, was used for the design of internal probes TSagHF436 (SEQ ID NO. 582) and TSagHF465 (SEQ ID NO. 583). The region of the amplicon selected for internal probes contained sequences unique and specific to *S. agalactiae*. SEQ ID NO. 583, the more specific probe, is labelled with fluorescein in 3', while SEQ ID NO. 582, the less discriminant probe, is labelled with CY5 in 5' and blocked in 3' with a phosphate group. However, since the FRET signal is only emitted if both probes are adjacently hybridized on the same target amplicon, detection is highly specific.

Real-time detection of PCR products using the LightCycler™ was carried out using 0.4 μ M of each primer (SEQ ID NO. 549-550), 0.2 μ M of each probe (SEQ ID NO. 582-583), 2.5 mM MgCl₂, BSA 450 μ g/ml, 1X PC2 Buffer (AB Peptides, St-Louis, MO), dNTP 0.2 mM (Pharmacia), 0.5 U KlenTaq1™ DNA polymerase (AB Peptides) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 0.7 μ l of genomic DNA sample in a final volume of 7 μ l using a LightCycler thermocycler (Idaho Technology). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 94 °C for initial denaturation, then forty cycles of three steps consisting of 0 second (this setting meaning the LightCycler will reach the target temperature and stay at it for its minimal amount of time) at 94 °C, 10 seconds at 64 °C, 20 seconds at 72 °C. Amplification was monitored during each annealing steps using the fluorescence ratio. The streptococcal species having close sequence homologies with the *tuf* sequence of *S. agalactiae* (*S. acidominimus*, *S. anginosus*, *S. bovis*, *S. dysgalactiae*, *S. equi*, *S. ferus*, *S. gordonii*, *S. intermedius*, *S. parasanguis*, *S. parauberis*, *S. salivarius*, *S. sanguis*, *S. suis*) as well as *S. agalactiae* were tested in the

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LightCycler with 0.07 ng of genomic DNA per reaction. Only *S. agalactiae* yielded an amplification signal, hence demonstrating that the assay is species-specific. With the LightCycler™ assay using the internal FRET probes, the detection limit for *S. agalactiae* was 1-2 genome copies of genomic DNA.

EXAMPLE 6:

Specific detection and identification of *Streptococcus agalactiae* using *atpD* sequences. As shown in Annex X, the comparison of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The primer design strategy is similar to the strategy described in the preceding Example except that *atpD* sequences were used in the alignment.

Four primers were selected, ASag42 (SEQ ID NO. 627), ASag52 (SEQ ID NO. 628), ASag206 (SEQ ID NO. 625) and ASag371 (SEQ ID NO. 626). The following combinations of these four primers give four amplicons; SEQ ID NO. 627 + SEQ ID NO. 625 = 190 bp, SEQ ID NO. 628 + SEQ ID NO. 625 = 180 bp, SEQ ID NO. 627 + SEQ ID NO. 626 = 355 bp, and SEQ ID NO. 628 + SEQ ID NO. 626 = 345 bp.

Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc) using 0.4 μ M of each primers pair, 2.5 mM MgCl₂, BSA 0.05 mM, 1X *taq* Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 1 μ l of genomic DNA sample in a final volume of 20 μ L. The optimal cycling conditions for maximum sensitivity and specificity were adjusted for each primer pair. Three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at the optimal annealing temperature specified below were followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing

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0.25 µg/ml of ethidium bromide. Since *atpD* sequences are relatively more specific than *tuf* sequences, only the most closely related species namely, the staphylococcal species listed in Table 9, were tested.

All four primer pairs only amplified the six *S. agalactiae* strains. With an annealing temperature of 63 °C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 625 had a sensitivity of 1-5 fg (equivalent to 1-2 genome copies). At 55 °C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 625 had a sensitivity of 2.5 fg (equivalent to 1 genome copy). At 60 °C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 626 had a sensitivity of 10 fg (equivalent to 4 genome copies). At 58 °C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 626 had a sensitivity of 2.5-5 fg (equivalent to 1-2 genome copies). This proves that all four primer pairs can detect *S. agalactiae* with high specificity and sensitivity. Together with Example 5, this example demonstrates that both *tuf* and *atpD* sequences are suitable and flexible targets for the identification of microorganisms at the species level. The fact that 4 different primer pairs based on *atpD* sequences led to efficient and specific amplification of *S. agalactiae* demonstrates that the challenge is to find target genes suitable for diagnostic purposes, rather than finding primer pairs from these target sequences.

EXAMPLE 7:

Development of a PCR assay for detection and identification of staphylococci at genus and species levels.

Materials and Methods

Bacterial strains. The specificity of the PCR assay was verified by using a panel of ATCC (America Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; German Collection of

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Microorganisms and Cell Cultures) reference strains consisting of 33 gram-negative and 47 gram-positive bacterial species (Table 12). In addition, 295 clinical isolates representing 11 different species of staphylococci from the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL) (Ste-Foy, Québec, Canada) were also tested to further validate the *Staphylococcus*-specific PCR assay. These strains were all identified by using (i) conventional methods or (ii) the automated MicroScan Autoscan-4 system equipped with the Positive BP Combo Panel Type 6 (Dade Diagnostics, Mississauga, Ontario, Canada). Bacterial strains from frozen stocks kept at -80 °C in brain heart infusion (BHI) broth containing 10% glycerol were cultured on sheep blood agar or in BHI broth (Quelab Laboratories Inc, Montréal, Québec, Canada).

PCR primers and internal probes. Based on multiple sequence alignments, regions of the *tuf* gene unique to staphylococci were identified. *Staphylococcus*-specific PCR primers TStaG422 (SEQ ID NO. 553) and TStaG765 (SEQ ID NO. 575) were derived from these regions (Annex XII). These PCR primers are displaced by two nucleotide positions compared to original *Staphylococcus*-specific PCR primers described in our patent publication WO98/20157 (SEQ ID NOs. 17 and 20 in the said patent publication). These modifications were done to ensure specificity and ubiquity of the primer pair, in the light of new *tuf* sequence data revealed in the present patent application for several additional staphylococcal species and strains.

Similarly, sequence alignment analysis were performed to design genus and species-specific internal probes (see Annexes XIII to XVI). Two internal probes specific for *Staphylococcus* (SEQ ID NOs. 605-606), five specific for *S. aureus* (SEQ ID NOs. 584-588), five specific for *S. epidermidis* (SEQ ID NO. 589-593), two specific for *S. haemolyticus* (SEQ ID NOs. 594-595), three specific for *S. hominis* (SEQ ID NOs. 596-598), four specific for *S. saprophyticus* (SEQ ID NOs. 599-601 and 695), and two specific for coagulase-negative *Staphylococcus* species including

S. epidermidis, *S. hominis*, *S. saprophyticus*, *S. auricularis*, *S. capitis*, *S. haemolyticus*, *S. lugdunensis*, *S. simulans*, *S. cohnii* and *S. warneri* (SEQ ID NOs. 1175-1176) were designed. The range of mismatches between the *Staphylococcus*-specific 371-bp amplicon and each of the 20-mer species-specific internal probes was from 1 to 5, in the middle of the probe when possible. No mismatches were present in the two *Staphylococcus*-specific probes for the 11 species analyzed: *S. aureus*, *S. auricularis*, *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. saprophyticus*, *S. simulans* and *S. warneri*. In order to verify the intra-specific sequence conservation of the nucleotide sequence, sequences were obtained for the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the species *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus*. The Oligo™ (version 5.0) primer analysis software (National Biosciences, Plymouth, Minn.) was used to confirm the absence of self-complementary regions within and between the primers or probes. When required, the primers contained inosines or degenerated nucleotides at one or more variable positions. Oligonucleotide primers and probes were synthesized on a model 394 DNA synthesizer (Applied Biosystems, Mississauga, Ontario, Canada). Detection of the hybridization was performed with the DIG-labeled dUTP incorporated during amplification with the *Staphylococcus*-specific PCR assay, and the hybridization signal was detected with a luminometer (Dynex Technologies) as described above in the section on luminescent detection of amplification products. Annexes XIII to XVI illustrate the strategy for the selection of several internal probes.

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA or from a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 bacteria per ml. One nanogram of genomic DNA or 1 μ l of the standardized bacterial suspension was transferred directly to a 19 μ l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM

MgCl₂, 0.2 µM (each) of the two *Staphylococcus* genus-specific primers (SEQ ID NOs. 553 and 575), 200 µM (each) of the four deoxynucleoside triphosphates (Pharmacia Biotech), 3.3 µg/µl bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), and 0.5 U *Taq* polymerase (Promega) coupled with *TaqStart*TM Antibody (Clontech). The PCR amplification was performed as follows: 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

For determination of the sensitivities of the PCR assays, two-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Results

Amplifications with the *Staphylococcus* genus-specific PCR assay. The specificity of the assay was assessed by performing 30-cycle and 40-cycle PCR amplifications with the panel of gram-positive (47 species from 8 genera) and gram-negative (33 species from 22 genera) bacterial species listed in Table 12. The PCR assay was able to detect efficiently 27 of 27 staphylococcal species tested in both 30-cycle and 40-cycle regimens. For 30-cycle PCR, all bacterial species tested other than staphylococci were negative. For 40-cycle PCR, *Enterococcus faecalis* and *Macrococcus caseolyticus* were slightly positive for the *Staphylococcus*-specific PCR assay. The other species tested remained negative. Ubiquity tests performed on a collection of 295 clinical isolates provided by the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), including *Staphylococcus aureus* (n=34), *S. auricularis* (n=2), *S. capitis* (n=19), *S. cohnii* (n=5), *S. epidermidis* (n=18), *S. haemolyticus*

(n=21), *S. hominis* (n=73), *S. lugdunensis* (n=17), *S. saprophyticus* (n=6), *S. simulans* (n=3), *S. warneri* (n=32) and *Staphylococcus* sp. (n=65), showed a uniform amplification signal with the 30-cycle PCR assays and a perfect relation between the genotype and classical identification schemes.

The sensitivity of the *Staphylococcus*-specific assay with 30-cycle and 40-cycle PCR protocols was determined by using purified genomic DNA from the 11 staphylococcal species previously mentioned. For PCR with 30 cycles, a detection limit of 50 copies of genomic DNA was consistently obtained. In order to enhance the sensitivity of the assay, the number of cycles was increased. For 40-cycle PCR assays, the detection limit was lowered to a range of 5-10 genome copies, depending on the staphylococcal species tested.

Hybridization between the *Staphylococcus*-specific 371-bp amplicon and species-specific or genus-specific internal probes. Inter-species polymorphism was sufficient to generate species-specific internal probes for each of the principal species involved in human diseases (*S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus*). In order to verify the intra-species sequence conservation of the nucleotide sequence, sequence comparisons were performed on the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the 5 principal staphylococcal species: *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus*. Results showed a high level of conservation of nucleotide sequence between different unrelated strains from the same species. This sequence information allowed the development of staphylococcal species identification assays using species-specific internal probes hybridizing to the 371-bp amplicon. These assays are specific and ubiquitous for those five staphylococcal species. In addition to the species-specific internal probes, the genus-specific internal probes were able to recognize all or most *Staphylococcus* species tested.

EXAMPLE 8:

Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis*. It is often useful for the clinician to be able to differentiate between two very closely related species of microorganisms. *Candida albicans* is the most important cause of invasive human mycose. In recent years, a very closely related species, *Candida dubliniensis*, was isolated in immunosuppressed patients. These two species are difficult to distinguish by classic biochemical methods. This example demonstrates the use of *tuf* sequences to differentiate *Candida albicans* and *Candida dubliniensis*. PCR primers SEQ ID NOs. 11-12, from previous patent publication WO98/20157, were selected for their ability to specifically amplify a *tuf* (elongation factor 1 alpha type) fragment from both species (see Annex XI for primer positions). Within this *tuf* fragment, a region differentiating *C. albicans* and *C. dubliniensis* by two nucleotides was selected and used to design two internal probes (see Annex XI for probe design, SEQ ID NOs. 577 and 578) specific for each species. Amplification of genomic DNA from *C. albicans* and *C. dubliniensis* was carried out using DIG-11-dUTP as described above in the section on chemiluminescent detection of amplification products. Internal probes SEQ ID NOs. 577 and 578 were immobilized on the bottom of individual microtiter plates and hybridization was carried out as described above in the above section on chemiluminescent detection of amplification products. Luminometer data showed that the amplicon from *C. albicans* hybridized only to probe SEQ ID NO. 577 while the amplicon from *C. dubliniensis* hybridized only to probe SEQ ID NO. 578, thereby demonstrating that each probe was species-specific.

EXAMPLE 9:

Specific identification of *Entamoeba histolytica*. Upon analysis of *tuf* (elongation factor 1 alpha) sequence data, it was possible to find four regions where

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Entamoeba histolytica sequences remained conserved while other parasitological and eukaryotic species have diverged. Primers TEntG38 (SEQ ID NO. 703), TEntG442 (SEQ ID NO. 704), TEntG534 (SEQ ID NO. 705), and TEntG768 (SEQ ID NO. 706) were designed so that SEQ ID NO. 703 could be paired with the three other primers. On PTC-200 thermocyclers (MJ Research), the cycling conditions for initial sensitivity and specificity testing were 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. The three primer pairs could detect the equivalent of less than 200 *E. histolytica* genome copies. Specificity was tested using 0.5 ng of purified genomic DNA from a panel of microorganisms including *Babesia bovis*, *Babesia microti*, *Candida albicans*, *Crithidia fasciculata*, *Leishmania major*, *Leishmania hertigi* and *Neospora caninum*. Only *E. histolytica* DNA could be amplified, thereby suggesting that the assay was species-specific.

EXAMPLE 10:

Sensitive identification of *Chlamydia trachomatis*. Upon analysis of *tuf* sequence data, it was possible to find two regions where *Chlamydia trachomatis* sequences remained conserved while other species have diverged. Primers Ctr82 (SEQ ID NO. 554) and Ctr249 (SEQ ID NO. 555) were designed. With the PTC-200 thermocyclers (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. The assay could detect the equivalent of 8 *C. trachomatis* genome copies. Specificity was tested with 0.1 ng of purified genomic DNA from a panel of microorganisms including 22 species commonly encountered

in the vaginal flora (*Bacillus subtilis*, *Bacteroides fragilis*, *Candida albicans*, *Clostridium difficile*, *Corynebacterium cervicis*, *Corynebacterium urealyticum*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus influenzae*, *Klebsiella oxytoca*, *Lactobacillus acidophilus*, *Peptococcus niger*, *Peptostreptococcus prevotii*, *Porphyromonas asaccharolytica*, *Prevotella melaninogenica*, *Propionibacterium acnes*, *Staphylococcus aureus*, *Streptococcus acidominimus*, and *Streptococcus agalactiae*). Only *C. trachomatis* DNA could be amplified, thereby suggesting that the assay was species-specific.

EXAMPLE 11:

Genus-specific detection and identification of enterococci. Upon analysis of *tuf* sequence data and comparison with the repertory of *tuf* sequences, it was possible to find two regions where *Enterococcus* sequences remained conserved while other genera have diverged (Annex XVII). Primer pair Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) was tested for its specificity by using purified genomic DNA from a panel of bacteria listed in Table 10. Using the PTC-200 thermocycler (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm. The 18 enterococcal species listed in Table 10 were all amplified efficiently. The only other species amplified were *Abiotrophia adiacens*, *Gemella haemolysans* and *Gemella morbillorum*, three gram-positive species. Sensitivity tested with several strains of *E. casseliflavus*, *E. faecium*, *E. faecalis*, *E. flavescens* and *E. gallinarum* and with one strain of each other *Enterococcus* species listed in Table 10 ranged from 1 to 10 copies of genomic DNA. The sequence variation

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within the 308-bp amplicon was sufficient so that internal probes could be used to speciate the amplicon and differentiate enterococci from *Abiotrophia adiacens*, *Gemella haemolysans* and *Gemella morbillorum*, thereby allowing to achieve excellent specificity. Species-specific internal probes were generated for each of the clinically important species, *E. faecalis* (SEQ ID NO. 1174), *E. faecium* (SEQ ID NO. 602), and the group including *E. casseliflavus*, *E. flavescens* and *E. gallinarum* (SEQ ID NO. 1122) (Annex XVIII). The species-specific internal probes were able to differentiate their respective *Enterococcus* species from all other *Enterococcus* species. These assays are sensitive, specific and ubiquitous for those five *Enterococcus* species.

EXAMPLE 12:

Identification of the major bacterial platelets contaminants using *tuf* sequences with a multiplex PCR test. Blood platelets preparations need to be monitored for bacterial contaminations. The *tuf* sequences of 17 important bacterial contaminants of platelets were aligned. As shown in Annex XIX, analysis of these sequences allowed the design of PCR primers. Since in the case of contamination of platelet concentrates, detecting all species (not just the more frequently encountered ones) is desirable, perfect specificity of primers was not an issue in the design. However, sensitivity is important. That is why, to avoid having to put too much degeneracy, only the most frequent contaminants were included in primer design, knowing that the selected primers would anyway be able to amplify more species than the 17 used in the design because they target highly conserved regions of *tuf* sequences. Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelet concentrates were chosen (oligos Tplaq 769 and Tplaq 991, respectively SEQ ID NOs. 636 and 637) thereby permitting the detection of these bacterial species. However, sensitivity was slightly deficient with staphylococci. To ensure maximal sensitivity in the detection of all the more frequent bacterial contaminants, a multiplex assay also including oligonucleotide

primers targetting the *Staphylococcus* genera (oligos Stag 422, SEQ ID NO. 553; and Stag 765, SEQ ID NO. 575) was developed. The bacterial species detected with the assay are listed in Table 14.

The primer pairs, oligos SEQ ID NO. 636 and SEQ ID NO. 637 that give an amplification product of 245 pb, and oligos SEQ ID NO. 553 and SEQ ID NO. 575 that give an amplification product of 368 pb, were used simultaneously in the multiplex PCR assay. Detection of these PCR products was made on the LightCycler thermocycler (Idaho Technology) using SYBR[®] Green I (Molecular Probe Inc.). SYBR[®] Green I is a fluorescent dye that binds specifically to double-stranded DNA.

Fluorogenic detection of PCR products with the LightCycler was carried out using 1.0 μ M of both Tplaq primers (SEQ ID NOs. 636-637) and 0.4 μ M of both TStaG primers (SEQ ID NOs. 553 and 575), 2.5 mM MgCl₂, BSA 7.5 μ M, dNTP 0.2 mM (Pharmacia), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 U *Taq* DNA polymerase (Boehringer Mannheim) coupled with TaqStart[™] antibody (Clontech), and 0.07 ng of genomic DNA sample in a final volume of 7 μ l. The optimal cycling conditions for maximum sensitivity and specificity were 1 minute at 94 °C for initial denaturation, then forty-five cycles of three steps consisting of 0 second at 95 °C, 5 seconds at 60 °C and 9 seconds at 72 °C. Amplification was monitored during each elongation cycle by measuring the level of SYBR[®] Green I. However, real analysis takes place after PCR. Melting curves are done for each sample and transformation of the melting peak allows determination of T_m. Thus primer-dimer and specific PCR product are discriminated. With this assay, all prominent bacterial contaminants of platelet concentrates listed in Annex XIX and Table 14 were detected. Sensitivity tests were performed on the 9 most frequent bacterial contaminants of platelets. The detection limit was less than 20 genome copies for *E. cloacae*, *B. cereus*, *S. choleraesuis* and *S. marcescens*; less than 15 genome copies for *P. aeruginosa*; and 2 to 3 copies were detected for *S. aureus*, *S.*

epidermidis, *E. coli* and *K. pneumoniae*. Further refinements of assay conditions should increase sensitivity levels.

EXAMPLE 13:

The resolving power of the *tuf* and *atpD* sequences databases is comparable to the biochemical methods for bacterial identification. The present gold standard for bacterial identification is mainly based on key morphological traits and batteries of biochemical tests. Here we demonstrate that the use of *tuf* and *atpD* sequences combined with simple phylogenetic analysis of databases formed by these sequences is comparable to the gold standard. In the process of acquiring data for the *tuf* sequences, we sequenced the *tuf* gene of a strain that was given to us labelled as *Staphylococcus hominis* ATCC 35982. That *tuf* sequence (SEQ ID NO. 192) was incorporated into the *tuf* sequences database and subjected to a basic phylogenetic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group). This analysis indicated that SEQ ID NO. 192 is not associated with other *S. hominis* strains but rather with the *S. warneri* strains. The ATCC 35982 strain was sent to the reference laboratory of the Laboratoire de santé publique du Québec (LSPQ). They used the classic identification scheme for staphylococci (Kloos and Schleifer, 1975., J. Clin. Microbiol. 1:82-88). Their results shown that although the colonial morphology could correspond to *S. hominis*, the more precise biochemical assays did not. These assays included discriminant mannitol, mannose and ribose acidification tests as well as rapid and dense growth in deep thioglycolate agar. The LSPQ report identified strain ATCC 35982 as *S. warneri* which confirms our database analysis. The same thing happened for *S. warneri* (SEQ ID NO. 187) which had initially been identified as *S. haemolyticus* by a routine clinical laboratory using a low resolving power automated system (MicroScan, AutoScan-4™). Again, the *tuf* and LSPQ analysis agreed on its identification as *S. warneri*. In numerous other instances, in the course of acquiring *tuf* and *atpD* sequence data from various species and genera,

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analysis of our *tuf* and/or *atpD* sequence databases permitted the exact identification of mislabelled or erroneously identified strains. These results clearly demonstrate the usefulness and the high resolving power of our sequence-based identification assays using the *tuf* and *atpD* sequences databases.

EXAMPLE 14:

Detection of group B streptococci from clinical specimens.

Introduction

Streptococcus agalactiae, the group B streptococcus (GBS), is responsible for a severe illness affecting neonate infants. The bacterium is passed from the healthy carrier mother to the baby during delivery. To prevent this infection, it is recommended to treat expectant mothers susceptible of carrying GBS in their vaginal/anal flora. Carrier status is often a transient condition and rigorous monitoring requires cultures and classic bacterial identification weeks before delivery. To improve the detection and identification of GBS we developed a rapid, specific and sensitive PCR test fast enough to be performed right at delivery.

Materials and Methods

GBS clinical specimens. A total of 66 duplicate vaginal/anal swabs were collected from 41 consenting pregnant women admitted for delivery at the Centre Hospitalier Universitaire de Québec, Pavillon Saint-François d'Assise following the CDC recommendations. The samples were obtained either before or after rupture of membranes. The swab samples were tested at the Centre de Recherche en Infectiologie de l'Université Laval within 24 hours of collection. Upon receipt, one swab was cut and then the tip of the swab was added to GNS selective broth for identification of group B streptococci (GBS) by the standard culture methods

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recommended by the CDC. The other swab was processed following the instruction of the IDI DNA extraction kit (Infectio Diagnostics (IDI) Inc.) prior to PCR amplification.

Oligonucleotides. PCR primers, Tsag340 (SEQ ID NO. 549) and Tsag552 (SEQ ID NO. 550) complementary to the regions of the *tuf* gene unique for GBS were designed based upon a multiple sequence alignment using our repertory of *tuf* sequences. Oligo primer analysis software (version 5.0) (National Biosciences) was used to analyse primers annealing temperature, secondary structure potential as well as mispriming and dimerization potential. The primers were synthesized using a model 391 DNA synthesizer (Applied Biosystems).

A pair of fluorescently labeled adjacent hybridization probes Sag465-F (SEQ ID NO. 583) and Sag436-C (SEQ ID NO. 582) were synthesized and purified by Operon Technologies. They were designed to meet the recommendations of the manufacturer (Idaho Technology) and based upon multiple sequence alignment analysis using our repertory of *tuf* sequences to be specific and ubiquitous for GBS. These adjacent probes, which are separated by one nucleotide, allow fluorescence resonance energy transfer (FRET), generating an increased fluorescence signal when both hybridized simultaneously to their target sequences. The probe SEQ ID NO. 583 was labeled with FITC in 3 prime while SEQ ID NO. 582 was labeled with Cy5 in 5 prime. The Cy5-labeled probes contained a 3'-blocking phosphate group to prevent extension of the probes during the PCR reactions.

PCR amplification. Conventional amplifications were performed either from 2 μ l of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 20 μ l PCR mixture contained 0.4 μ M of each GBS-specific primer (SEQ ID NOs. 549-550), 200 μ M of each deoxyribonucleotide (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl₂, 3.3 mg/ml bovine serum albumin (BSA) (Sigma), and 0.5 U of *Taq* polymerase (Promega) combined with the TaqStartTM antibody (Clontech). The TaqStartTM antibody, which is a neutralizing monoclonal antibody of *Taq* DNA

polymerase, was added to all PCR reactions to enhance the efficiency of the amplification. The PCR mixtures were subjected to thermal cycling (3 min at 95 °C and then 40 cycles of 1 s at 95 °C, and 30 s at 62 °C with a 2-min final extension at 72 °C) with a PTC-200 DNA Engine thermocycler (MJ research). The PCR-amplified reaction mixture was resolved by agarose gel electrophoresis.

The LightCycler™ PCR amplifications were performed with 1 µl of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 10µl amplification mixture consisted of 0.4 µM each GBS-specific primer (SEQ ID NOs. 549-550), 200 µM each dNTP, 0.2 µM each fluorescently labeled probe (SEQ ID NOs. 582-583), 300 µg/ml BSA (Sigma), and 1 µl of 10x PC2 buffer (containing 50 mM Tris-HCl (pH 9.1), 16 mM ammonium sulfate, 3.5 mM Mg²⁺, and 150 µg/ml BSA) and 0.5 U KlenTaq1™ (AB Peptides) coupled with TaqStart™ antibody (Clontech). KlenTaq1™ is a highly active and more heat-stable DNA polymerase without 5'-exonuclease activity. This prevents hydrolysis of hybridized probes by the 5' to 3' exonuclease activity. A volume of 7 µl of the PCR mixture was transferred into a composite capillary tube (Idaho Technology). The tubes were then centrifuged to move the reaction mixture to the tips of the capillaries and then cleaned with optical-grade methanol. Subsequently the capillaries were loaded into the carousel of a LC32 LightCycler™ (Idaho Technology), an instrument that combines rapid-cycle PCR with fluorescence analysis for continuous monitoring during amplification. The PCR reaction mixtures were subjected to a denaturation step at 94 °C for 3 min followed by 45 cycles of 0 s at 94 °C, 20 s at 64 °C and 10 s at 72 °C with a temperature transition rate of 20 °C/s. Fluorescence signals were obtained at each cycle by sequentially positioning each capillary on the carousel at the focus of optical elements affiliated to the built-in fluorimeter for 100 milliseconds. Complete amplification and analysis required about 35 min.

Specificity and sensitivity tests. The specificity of the conventional and LightCycler™ PCR assays was verified by using purified genomic DNA (0.1 ng/reaction) from a battery of ATCC reference strains representing 35 clinically

relevant gram-positive species (*Abiotrophia defectiva* ATCC 49176, *Bifidobacterium breve* ATCC 15700, *Clostridium difficile* ATCC 9689, *Corynebacterium urealyticum* ATCC 43042, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus durans* ATCC 19432, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus gallinarum* ATCC 49573, *Enterococcus raffinosus* ATCC 49427, *Lactobacillus reuteri* ATCC 23273, *Lactococcus lactis* ATCC 19435, *Listeria monocytogenes* ATCC 15313, *Peptococcus niger* ATCC 27731, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus prevotii* ATCC 9321, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus agalactiae* ATCC 27591, *Streptococcus anginosus* ATCC 33397, *Streptococcus bovis* ATCC 33317, *Streptococcus constellatus* ATCC 27823, *Streptococcus dysgalactiae* ATCC 43078, *Streptococcus gordonii* ATCC 10558, *Streptococcus mitis* ATCC 33399, *Streptococcus mutans* ATCC 25175, *Streptococcus oralis* ATCC 35037, *Streptococcus parauberis* ATCC 6631, *Streptococcus pneumoniae* ATCC 6303, *Streptococcus pyogenes* ATCC 19615, *Streptococcus salivarius* ATCC 7073, *Streptococcus sanguinis* ATCC 10556, *Streptococcus uberis* ATCC 19436). These microbial species included 15 species of streptococci and many members of the normal vaginal and anal floras. In addition, 40 GBS isolates of human origin, whose identification was confirmed by a latex agglutination test (Streptex, Murex), were also used to evaluate the ubiquity of the assay.

For determination of the sensitivities (i.e., the minimal number of genome copies that could be detected) for conventional and LightCycler™ PCR assays, serial 10-fold or 2-fold dilutions of purified genomic DNA from 5 GBS ATCC strains were used.

Results

Evaluation of the GBS-specific conventional and LightCycler™ PCR assays. The specificity of the two assays demonstrated that only DNAs from GBS

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strains could be amplified. Both PCR assays did not amplify DNAs from any other bacterial species tested including 14 streptococcal species other than GBS as well as phylogenetically related species belonging to the genera *Enterococcus*, *Peptostreptococcus* and *Lactococcus*. Important members of the vaginal or anal flora, including coagulase-negative staphylococci, *Lactobacillus* sp., and *Bacteriodes* sp. were also negative with the GBS-specific PCR assay. The LightCycler™ PCR assays detected only GBS DNA by producing an increased fluorescence signal which was interpreted as a positive PCR result. Both PCR methods were able to amplify all of 40 GBS clinical isolates, showing a perfect correlation with the phenotypic identification methods.

The sensitivity of the assay was determined by using purified genomic DNA from the 5 ATCC strains of GBS. The detection limit for all of these 5 strains was one genome copy of GBS. The detection limit of the assay with the LightCycler™ was 3.5 fg of genomic DNA (corresponding to 1-2 genome copies of GBS). These results confirmed the high sensitivity of our GBS-specific PCR assay.

Direct Detection of GBS from vaginal/anal specimens. Among 66 vaginal/anal specimens tested, 11 were positive for GBS by both culture and PCR. There was one sample positive by culture only. The sensitivity of both PCR methods with vaginal/anal specimens for identifying colonization status in pregnant women at delivery was 91.7% when compared to culture results. The specificity and positive predictive values were both 100% and the negative predictive value was 97.8%. The time for obtaining results was approximately 45 min for LightCycler™ PCR, approximately 100 min for conventional PCR and 48 hours for culture.

Conclusion

We have developed two PCR assays (conventional and LightCycler™) for the detection of GBS, which are specific (i.e., no amplification of DNA from a variety of bacterial species other than GBS) and sensitive (i.e., able to detect around 1

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genome copy for several reference ATCC strains of GBS). Both PCR assays are able to detect GBS directly from vaginal/anal specimens in a very short turnaround time. Using the real-time PCR assay on LightCycler™, we can detect GBS carriage in pregnant women at delivery within 45 minutes.

EXAMPLE 15:

Simultaneous detection and identification of *Streptococcus pyogenes* and its pyrogenic exotoxin A. The rapid detection of *Streptococcus pyogenes* and of its pyrogenic exotoxin A is of clinical importance. We developed a multiplex assay which permits the detection of strains of *S. pyogenes* carrying the pyrogenic toxin A gene, which is associated with scarlet fever and other pathologies. In order to specifically detect *S. pyogenes*, nucleotide sequences of the pyrrolidone carboxyl peptide (pcp) gene were aligned to design PCR primers Spy291 (SEQ ID NO. 1211) and Spy473 (SEQ ID NO. 1210). Next, we designed primers for the specific detection of the pyrogenic exotoxin A. Nucleotide sequences of the *speA* gene, carried on the bacteriophage T12, were aligned as shown in Annex XXIII to design PCR primers Spytx814 (SEQ ID NO. 994) and Spytx 927 (SEQ ID NO. 995).

The primer pairs: oligos SEQ ID NOs. 1210-1211, yielding an amplification product of 207 bp, and oligos SEQ ID NOs. 994-995, yielding an amplification product of 135 bp, were used in a multiplex PCR assay.

PCR amplification was carried out using 0.4 μ M of both pairs of primers, 2.5 mM MgCl₂, BSA 0.05 μ M, dNTP 0.2 μ M (Pharmacia), 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), and 1 μ l of genomic DNA sample in a final volume of 20 μ l. PCR amplification was performed using a PTC-200 thermal cycler (MJ Research). The optimal cycling conditions for maximum specificity and sensitivity were 3 minutes at 94 °C for

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initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 63 °C, followed by a final step of 2 minutes at 72 °C. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

The detection limit was less than 5 genome copies for both *S. pyogenes* and its pyrogenic exotoxin A. The assay was specific for pyrogenic exotoxin A-producing *S. pyogenes*: strains of the 27 other species of *Streptococcus* tested, as well as 20 strains of various gram-positive and gram-negative bacterial species were all negative.

A similar approach was used to design an alternative set of *speA*-specific primers (SEQ ID NOs. 996 to 998, see Annex XXIV). In addition, another set of primers based on the *tuf* gene (SEQ ID NOs. 999 to 1001, see Annex XXV) could be used to specifically detect *Streptococcus pyogenes*.

EXAMPLE 16:

Real-time detection and identification of Shiga toxin-producing bacteria. Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* cause bloody diarrhea. Currently, identification relies mainly on the phenotypic identification of *S. dysenteriae* and *E. coli* serotype O157:H7. However, other serotypes of *E. coli* are increasingly found to be producers of type 1 and/or type 2 Shiga toxins. Two pairs of PCR primers targeting highly conserved regions present in each of the Shiga toxin genes *stx*₁ and *stx*₂ were designed to amplify all variants of those genes (see Annexes XXVI and XXVII). The first primer pair, oligonucleotides 1SLT224 (SEQ ID NO. 1081) and 1SLT385 (SEQ ID NO. 1080), yields an amplification product of 186 bp from the *stx*₁ gene. For this amplicon, the 1SLTB1-Fam (SEQ ID NO. 1084) molecular beacon was designed for the specific detection of *stx*₁

using the fluorescent label 6-carboxy-fluorescein. The 1SltS1-FAM (SEQ ID NO. 2012) molecular scorpion was also designed as an alternate way for the specific detection of *stx*₁. A second pair of PCR primers, oligonucleotides 2SLT537 (SEQ ID NO. 1078) and 2SLT678b (SEQ ID NO. 1079), yields an amplification product of 160 bp from the *stx*₂ gene. Molecular beacon 2SLTB1-Tet (SEQ ID NO. 1085) was designed for the specific detection of *stx*₂ using the fluorescent label 5-tetrachloro-fluorescein. Both primer pairs were combined in a multiplex PCR assay.

PCR amplification was carried out using 0.8 μ M of primer pair SEQ ID NOs. 1080-1081, 0.5 μ M of primer pair SEQ ID NOs. 1078-1079, 0.3 μ M of each molecular beacon, 8 mM MgCl₂, 490 μ g/mL BSA, 0.2 mM dNTPs (Pharmacia), 50 mM Tris-HCl, 16 mM NH₄SO₄, 1X TaqMaster (Eppendorf), 2.5 U KlenTaq1 DNA polymerase (AB Peptides) coupled with TaqStartTM antibody (Clontech Laboratories Inc.), and 1 μ l of genomic DNA sample in a final volume of 25 μ l. PCR amplification was performed using a SmartCycler thermal cycler (Cepheid). The optimal cycling conditions for maximum sensitivity and specificity were 60 seconds at 95 °C for initial denaturation, then 45 cycles of three steps consisting of 10 seconds at 95 °C, 15 seconds at 56 °C and 5 seconds at 72 °C. Detection of the PCR products was made in real-time by measuring the fluorescent signal emitted by the molecular beacon when it hybridizes to its target at the end of the annealing step at 56 °C.

The detection limit was the equivalent of less than 5 genome copies. The assay was specific for the detection of both toxins, as demonstrated by the perfect correlation between PCR results and the phenotypic characterization performed using antibodies specific for each Shiga toxin type. The assay was successfully performed on several Shiga toxin-producing strains isolated from various geographic areas of the world, including 10 O157:H7 *E. coli*, 5 non-O157:H7 *E. coli* and 4 *S. dysenteriae*.

EXAMPLE 17:

Development of a PCR assay for the detection and identification of staphylococci at genus and species levels and its associated *mecA* gene. The *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) were used in multiplex with the *mecA*-specific PCR primers and the *S. aureus*-specific primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 for *mecA* and SEQ ID NOs. 152 and 153 for *S.aureus* in the said patent). Sequence alignment analysis of 10 publicly available *mecA* gene sequences allowed to design an internal probe specific to *mecA* (SEQ ID NO. 1177). An internal probe was also designed for the *S. aureus*-specific amplicon (SEQ ID NO 1234). PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μ M (each) of the two *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μ M (each) of the *mecA*-specific primers and 0.4 μ M (each) of the *S. aureus*-specific primers were used in the PCR mixture. The specificity of the multiplex assay with 40-cycle PCR protocols was verified by using purified genomic DNA from five methicillin-resistant and fifteen methicillin-sensitive staphylococcal strains. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from twenty-three methicillin-resistant and twenty-eight methicillin-sensitive staphylococcal strains. The detection limit was 2 to 10 genome copies of genomic DNA, depending on the staphylococcal species tested. Furthermore, the *mecA*-specific internal probe, the *S. aureus*-specific internal probe and the coagulase-negative staphylococci-specific internal probe (described in Example 7) were able to recognize twenty-three methicillin-resistant staphylococcal strains and twenty-eight methicillin-sensitive staphylococcal strains with high sensitivity and specificity.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1232 for detection of the *S. aureus*-specific amplicon, SEQ ID NO. 1233 for detection of coagulase-negative staphylococci and SEQ ID NO. 1231 for detection of *mecA*.

Alternatively, a multiplex PCR assay containing the *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) and the *mecA*-specific PCR primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were developed. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μ M (each) of the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μ M (each) of the *mecA*-specific primers described in our assigned US patent no. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were used in the PCR mixture. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from two methicillin-resistant and five methicillin-sensitive staphylococcal strains. The detection limit was 2 to 5 copies of genomic DNA, depending on the staphylococcal species tested. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with two strains of methicillin-resistant *S. aureus*, two strains of methicillin-sensitive *S. aureus* and seven strains of methicillin-sensitive coagulase-negative staphylococci. The *mecA*-specific internal probe (SEQ ID NO. 1177) and the *S. aureus*-specific internal probe (SEQ ID NO. 587) described in Example 7 were able to recognize all the strains with high specificity showing a perfect correlation with susceptibility to methicillin. The sensitivity of the PCR assay coupled with capture-probe hybridization was tested with one strain of methicillin-resistant *S. aureus*. The detection limit was around 10 copies of genomic DNA.

EXAMPLE 18:Sequencing of *pbp1a*, *pbp2b* and *pbp2x* genes of *Streptococcus pneumoniae*.

Penicillin resistance in *Streptococcus pneumoniae* involves the sequential alteration of up to five penicillin-binding proteins (PBPs) 1A, 1B, 2A, 2X and 2B in such a way that their affinity is greatly reduced toward the antibiotic molecule. The altered PBP genes have arisen as the result of interspecies recombination events from related streptococcal species. Among the PBPs usually found in *S. pneumoniae*, PBPs 1A, 2B, and 2X play the most important role in the development of penicillin resistance. Alterations in PBP 2B and 2X mediate low-level resistance to penicillin while additional alterations in PBP 1A play a significant role in full penicillin resistance.

In order to generate a database for *pbp* sequences that can be used for design of primers and/or probes for the specific and ubiquitous detection of β -lactam resistance in *S. pneumoniae*, *pbp1a*, *pbp2b* and *pbp2x* DNA fragments sequenced by us or selected from public databases (GenBank and EMBL) from a variety of *S. pneumoniae* strains were used to design oligonucleotide primers. This database is essential for the design of specific and ubiquitous primers and/or probes for detection of β -lactam resistance in *S. pneumoniae* since the altered PBP 1A, PBP 2B and PBP 2X of β -lactam resistant *S. pneumoniae* are encoded by mosaic genes with numerous sequence variations among resistant isolates. The PCR primers were located in conserved regions of *pbp* genes and were able to amplify *pbp1a*, *pbp2b*, and *pbp2x* sequences of several strains of *S. pneumoniae* having various levels of resistance to penicillin and third-generation cephalosporins. Using primer pairs SEQ ID NOs. 1125 and 1126, SEQ ID NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, it was possible to amplify and determine *pbp1a* sequences SEQ ID NOs. 1004-1018, 1648, 2056-2060 and 2062-2064, *pbp2b* sequences SEQ ID NOs. 1019-1033, and *pbp2x* sequences SEQ ID NOs. 1034-1048. Six other PCR primers

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(SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) were also designed and used to complete the sequencing of *pbp1a*, *pbp2b* and *pbp2x* amplification products. The described primers (SEQ ID NOs. 1125 and 1126, SEQ ID NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) represent a powerful tool for generating new *pbp* sequences for design of primers and/or probes for detection of β -lactam resistance in *S. pneumoniae*.

EXAMPLE 19:

Sequencing of *hexA* genes of *Streptococcus* species. The *hexA* sequence of *S. pneumoniae* described in our assigned US patent no. 5,994,066 (SEQ ID NO. 31 in the said patent, SEQ ID NO. 1183 in the present application) allowed the design of a PCR primer (SEQ ID NO. 1182) which was used with primer Spn1401 described in our assigned US patent no. 5,994,066 (SEQ ID NO. 156 in the said patent, SEQ ID NO. 1179 in the present application) to generate a database for *hexA* sequences that can be used to design primers and/or probes for the specific identification and detection of *S. pneumoniae* (Annex XLII). Using primers SEQ ID NO. 1179 and SEQ ID NO. 1182 (Annex XLII), it was possible to amplify and determine the *hexA* sequence from *S. pneumoniae* (4 strains) (SEQ ID NOs. 1184-1187), *S. mitis* (three strains) (SEQ ID NOs. 1189-1191) and *S. oralis* (SEQ ID NO. 1188).

EXAMPLE 20:

Development of multiplex PCR assays coupled with capture probe hybridization for the detection and identification of *Streptococcus pneumoniae* and its penicillin resistance genes.

Two different assays were developed to identify *S. pneumoniae* and its susceptibility to penicillin.

ASSAY I:

Bacterial strains. The specificity of the multiplex PCR assay was verified by using a panel of ATCC (American Type Culture Collection) reference strains consisting of 33 gram-negative and 67 gram-positive bacterial species (Table 13). In addition, a total of 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis* from the American Type Culture Collection, the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), (Ste-Foy, Québec, Canada), the Laboratoire de santé publique du Québec, (Sainte-Anne-de-Bellevue, Québec, Canada), the Sunnybrook and Women's College Health Sciences Centre (Toronto, Canada), the Infectious Diseases Section, Department of Veterans Affairs Medical Center, (Houston, USA) were also tested to further validate the *Streptococcus pneumoniae*-specific PCR assay. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

PCR primers and internal probes. The analysis of *hexA* sequences from a variety of streptococcal species from the publicly available *hexA* sequence and from the database described in Example 19 (SEQ ID NOs. 1184-1191) allowed the selection of a PCR primer specific to *S. pneumoniae*, SEQ ID NO. 1181. This primer was used with the *S. pneumoniae*-specific primer SEQ ID NO. 1179 to generate an amplification product of 241 bp (Annex XLII). The PCR primer SEQ ID NO. 1181 is located 127 nucleotides downstream on the *hexA* sequence compared to the original *S. pneumoniae*-specific PCR primer Spn1515 described in our assigned US patent no. 5,994,066 (SEQ ID NO. 157 in the said patent). These modifications were done to ensure the design of the *S. pneumoniae*-specific internal probe according to the new *hexA* sequences of several streptococcal species from the database described in Example 19 (SEQ ID NOs. 1184-1191).

The analysis of *pbp1a* sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the identification of amino acid substitutions Ile-459 to Met and Ser-462 to Ala that occur in isolates with high-level penicillin resistance (MICs $\geq 1\mu\text{g/ml}$), and amino acid substitutions Ser-575 to Thr, Gln-576 to Gly and Phe-577 to Tyr that are common to all penicillin-resistant isolates with MICs $\geq 0.25\mu\text{g/ml}$. As shown in Annex XXXI, PCR primer pair SEQ ID NOs. 1130 and 1131 were designed to detect high-level penicillin resistance (MICs $\geq 1\mu\text{g/ml}$), whereas PCR primer pair SEQ ID NOs. 1129 and 1131 were designed to detect intermediate- and high-level penicillin resistance (MICs $\geq 0.25\mu\text{g/ml}$).

The analysis of *hexA* sequences from the publicly available *hexA* sequence and from the database described in Example 19 allowed the design of an internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) (Annex XLII). The range of mismatches between the *S. pneumoniae*-specific 241-bp amplicon was from 2 to 5, in the middle of the 19-bp probe. The analysis of *pbp1a* sequences from public databases and from the database described in Example 18 allowed the design of five internal probes containing all possible mutations to detect the high-level penicillin resistance 383-bp amplicon (SEQ ID NOs. 1197, 1217-1220). Alternatively, two other internal probes (SEQ ID NOs. 2024-2025) can also be used to detect the high-level penicillin resistance 383-bp amplicon. Five internal probes containing all possible mutations to detect the 157-bp amplicon which includes intermediate- and high-level penicillin resistance were also designed (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). Design and synthesis of primers and probes, and detection of the probe hybridization were performed as described in Example 7. Annex XXXI illustrates one of the internal probe for detection of the high-level penicillin resistance 383-bp amplicon (SEQ ID NO. 1197) and one of the internal probe for detection of the intermediate- and high-level penicillin resistance 157-bp amplicon (SEQ ID NO. 1193).

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μ l of genomic DNA at 0.1 ng/ μ l, or 1 μ l of a bacterial lysate, was transferred to a 19 μ l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (H 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 0.1 μ M (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.2 μ M of primer SEQ ID NO. 1129, 0.7 μ M of primer SEQ ID NO. 1131, and 0.6 μ M of primer SEQ ID NO. 1130, 0.05 mM bovine serum albumin (BSA), and 0.5 U *Taq* polymerase (Promega) coupled with TaqStart™ antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

For determination of the sensitivity of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Capture probe hybridization. The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio ≥ 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

Results

Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17

genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. There was a perfect correlation between PCR and standard susceptibility testing for 33 penicillin-sensitive isolates. Among 12 *S. pneumoniae* isolates with intermediate penicillin resistance based on susceptibility testing, 11 had intermediate resistance based on PCR, but one *S. pneumoniae* isolate with penicillin MIC of 0.25 µg/ml showed a high-level penicillin resistance based on genotyping. Among 53 isolates with high-level penicillin resistance based on susceptibility testing, 51 had high-level penicillin resistance based on PCR but two isolates with penicillin MIC > 1 µg/ml showed an intermediate penicillin resistance based on genotyping. In general, there was a good correlation between the genotype and classical culture method for bacterial identification and susceptibility testing.

The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneumoniae* strains but did not hybridize to the *S. mitis* and *S. oralis* amplicons. The five internal probes specific to the high-level resistance amplicon (SEQ ID NOs. 1197, 1217-1220) detected all amplification patterns corresponding to high-level resistance. The two *S. pneumoniae* strains with penicillin MIC > 1 µg/ml that showed an intermediate penicillin resistance based on PCR amplification were also intermediate resistance based on probe hybridization. Similarly, among 12 strains

with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with the five internal probes specific to the intermediate and high-level resistance amplicon (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). The strain described above having a penicillin MIC of 0.25 µg/ml which was high-level penicillin resistance based on PCR amplification was also high-level resistance based on probe hybridization. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae*.

ASSAY II:

Bacterial strains. The specificity of the multiplex PCR assay was verified by using the same strains as those used for the development of Assay I. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

PCR primers and internal probes. The analysis of *pbpla* sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the design of two primers located in the constant region of *pbpla*. PCR primer pair (SEQ ID NOs. 2015 and 2016) was designed to amplify a 888-bp variable region of *pbpla* from all *S. pneumoniae* strains. A series of internal probes were designed for identification of the *pbpla* mutations associated with penicillin resistance in *S. pneumoniae*. For detection of high-level penicillin resistance (MICs $\geq 1\mu\text{g/ml}$), three internal probes were designed (SEQ ID NOs. 2017-2019). Alternatively, ten other internal probes were designed that can also be used for detection of high-level resistance within the 888-bp *pbpla* amplicon: (1) three internal probes for identification of the amino acid substitutions Thr-371 to Ser or Ala within the motif S370TMK (SEQ ID NOs. 2031-2033); (2) two internal probes for detection

of the amino acid substitutions Ile-459 to Met and Ser-462 to Ala near the motif S428RN (SEQ ID NOs. 1135 and 2026); (3) two internal probes for identification of the amino acid substitutions Asn-443 to Asp (SEQ ID NOs. 1134 and 2027); and (4) three internal probes for detection of all sequence variations within another region (SEQ ID NOs. 2028-2030). For detection of high-level and intermediate penicillin resistance (MICs ≥ 0.25 $\mu\text{g/ml}$), four internal probes were designed (SEQ ID NOs. 2020-2023). Alternatively, six other internal probes were designed for detection of the four consecutive amino acid substitutions T574SQF to A574TGY near the motif K557TG (SEQ ID NOs. 2034-2039) that can also be used for detection of intermediate- and high-level resistance within the 888-bp *pbp1a* amplicon.

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μl of genomic DNA at 0.1 ng/ μl , or 1 μl of a bacterial lysate, was transferred to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl_2 , 0.08 μM (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.4 μM of the *pbp1a*-specific primer SEQ ID NO. 2015, 1.2 μM of *pbp1a*-specific primer SEQ ID NO. 2016, 0.05 mM bovine serum albumin (BSA), and 0.5 U *Taq* polymerase (Promega) coupled with TaqStartTM antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

For determination of the sensitivities of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Capture probe hybridization. The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates as described for Assay I.

Results

Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17 genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. All the above *S. pneumoniae* strains produced the 888-bp amplicon corresponding to *pbp1a* and the 241-bp fragment corresponding to *hexA*.

The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneumoniae* strains but did not hybridize to the *S. mitis* and *S. oralis* amplicons. The three internal probes (SEQ ID NOs 2017-2019) specific to high-level resistance detected all the 43 strains with high-level penicillin resistance based on susceptibility testing. Among 12 isolates with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with 4 internal probes (SEQ ID NOs. 2020-2023) and one strain

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having penicillin MIC of 0.25 µg/ml was misclassified as high-level penicillin resistance. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae*.

EXAMPLE 21:

Sequencing of the vancomycin resistance *vanA*, *vanC1*, *vanC2* and *vanC3* genes.

The publicly available sequences of the *vanH-vanA-vanX-vanY* locus of transposon Tn1546 from *E. faecalis*, *vanC1* sequence from one strain of *E. gallinarum*, *vanC2* and *vanC3* sequences from a variety of *E. casseliflavus* and *E. flavescens* strains, respectively, allowed the design of PCR primers able to amplify the *vanA*, *vanC1*, *vanC2* and *vanC3* sequences of several *Enterococcus* species. Using primer pairs van6877 and van9106 (SEQ ID NOs. 1150 and 1155), vanC1-122 and vanC1-1315 (SEQ ID NOs. 1110 and 1109), and vanC2C3-1 and vanC2C3-1064 (SEQ ID NOs. 1108 and 1107), it was possible to amplify and determine *vanA* sequences SEQ ID NOs. 1049-1057, *vanC1* sequences SEQ ID NOs. 1058-1059, *vanC2* sequences SEQ ID NOs. 1060-1063 and *vanC3* sequences SEQ ID NOs. 1064-1066, respectively. Four other PCR primers (SEQ ID NOs. 1151-1154) were also designed and used to complete the sequencing of *vanA* amplification products.

EXAMPLE 22:

Development of a PCR assay for the detection and identification of enterococci at genus and species levels and its associated resistance genes *vanA* and *vanB*. The comparison of *vanA* and *vanB* sequences revealed conserved regions allowing the design of PCR primers specific to both *vanA* and *vanB* sequences (Annex XXXVIII). The PCR primer pair vanAB459 and vanAB830R (SEQ ID NOs. 1112 and 1111) was used in multiplex with the *Enterococcus*-specific primers Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) described in Example

11. Sequence alignment analysis of *vanA* and *vanB* sequences revealed regions suitable for the design of internal probes specific to *vanA* (SEQ ID NO. 1170) and *vanB* (SEQ ID NO. 1171). PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 11. The optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62°C, plus a terminal extension at 72 °C for 2 minutes. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 nanogram of purified genomic DNA from a panel of bacteria listed in Table 10. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of *E. casseliflavus*, eight strains of *E. gallinarum*, two strains of *E. flavescens*, two vancomycin-resistant strains of *E. faecalis* and one vancomycin-sensitive strain of *E. faecalis*, three vancomycin-resistant strains of *E. faecium*, one vancomycin-sensitive strain of *E. faecium* and one strain of each of the other enterococcal species listed in Table 10. The detection limit was 1 to 10 copies of genomic DNA, depending on the enterococcal species tested. The *vanA*- and *vanB*-specific internal probes (SEQ ID NOs. 1170 and 1171), as well as the *E. faecalis*- and *E. faecium*-specific internal probes (SEQ ID NOs. 1174 and 602) and the internal probe specific to the group including *E. casseliflavus*, *E. gallinarum* and *E. flavescens* (SEQ ID NO. 1122) described in Example 11, were able to recognize vancomycin-resistant enterococcal species with high sensitivity, specificity and ubiquity showing a perfect correlation between the genotypic and phenotypic analysis.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1236 for the detection of *E. faecalis*, SEQ ID NO. 1235 for the detection of *E. faecium*, SEQ ID NO. 1240 for the detection of *vanA*, and SEQ ID NO. 1241 for the detection of *vanB*.

EXAMPLE 23:

Development of a multiplex PCR assay for detection and identification of vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium* and the group including *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*. The analysis of *vanA* and *vanB* sequences revealed conserved regions allowing design of a PCR primer pair (SEQ ID NOs. 1089 and 1090) specific to *vanA* sequences (Annex XXVIII) and a PCR primer pair (SEQ ID NOs. 1095 and 1096) specific to *vanB* sequences (Annex XXIX). The *vanA*-specific PCR primer pair (SEQ ID NOs. 1089 and 1090) was used in multiplex with the *vanB*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent). The comparison of *vanC1*, *vanC2* and *vanC3* sequences revealed conserved regions allowing design of PCR primers (SEQ ID NOs. 1101 and 1102) able to generate a 158-bp amplicon specific to the group including *E. gallinarum*, *E. casseliflavus* and *E. flavescens* (Annex XXX). The *vanC*-specific PCR primer pair (SEQ ID NOs. 1101 and 1102) was used in multiplex with the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. The *vanA*-specific PCR primer pair (SEQ ID NOs. 1089 and 1090), the *vanB*-specific primer pair (SEQ ID NOs. 1095 and 1096) and the *vanC*-specific primer pair (SEQ ID NOs. 1101 and 1102) were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of 5 vancomycin-

sensitive *Enterococcus* species, 3 vancomycin-resistant *Enterococcus* species, 13 other gram-positive bacteria and one gram-negative bacterium. Specificity tests were performed with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication) and with the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) on a panel of 37 gram-positive bacterial species. All *Enterococcus* strains were amplified with high specificity showing a perfect correlation between the genotypic and phenotypic analysis. The sensitivity of the assays was determined for several strains of *E. gallinarum*, *E. casseliflavus*, *E. flavescens* and vancomycin-resistant *E. faecalis* and *E. faecium*. Using each of the *E. faecalis*- and *E. faecium*-specific PCR primer pairs as well as *vanA*-, *vanB*- and *vanC*-specific PCR primers used alone or in multiplex as described above, the sensitivity ranged from 1 to 10 copies of genomic DNA.

The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1238 for the detection of *E. faecalis*, SEQ ID NO. 1237 for the detection of *E. faecium*, SEQ ID NO. 1239 for the detection of *vanA*, and SEQ ID NO. 1241 for the detection of *vanB*.

Alternatively, another PCR assay was developed for the detection of vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis*. This assay included two multiplex: (1) the first multiplex contained the *vanA*-specific primer pair (SEQ ID NOs. 1090-1091) and the *vanB*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent), and (2) the second multiplex contained the *E. faecalis*-specific PCR primer pair described in our assigned US patent 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1

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and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94 °C, followed by forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, plus a terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 µg/ml of ethidium bromide. The two multiplexes were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of two vancomycin-sensitive *E. faecalis* strains, two vancomycin-resistant *E. faecalis* strains, two vancomycin-sensitive *E. faecium* strains, two vancomycin-resistant *E. faecium* strains, 16 other enterococcal species and 31 other gram-positive bacterial species. All the *E. faecium* and *E. faecalis* strains were amplified with high specificity showing a perfect correlation between the genotypic analysis and the susceptibility to glycopeptide antibiotics (vancomycin and teicoplanin). The sensitivity of the assay was determined for two vancomycin-resistant *E. faecalis* strains and two vancomycin-resistant *E. faecium* strains. The detection limit was 5 copies of genomic DNA for all the strains.

This multiplex PCR assay was coupled with capture-probe hybridization. Four internal probes were designed: one specific to the *vanA* amplicon (SEQ ID NO. 2292), one specific to the *vanB* amplicon (SEQ ID NO. 2294), one specific to the *E. faecalis* amplicon (SEQ ID NO. 2291) and one specific to the *E. faecium* amplicon (SEQ ID NO. 2287). Each of the internal probes detected their specific amplicons with high specificity and sensitivity.

EXAMPLE 24:

Universal amplification involving the EF-G (*fusA*) subdivision of *tuf* sequences. As shown in Figure 3, primers SEQ ID NOs. 1228 and 1229 were designed to amplify the region between the end of *fusA* and the beginning of *tuf* genes in the *str* operon. Genomic DNAs from a panel of 35 strains were tested for PCR amplification with those primers. In the initial experiment, the following strains showed a positive

result: *Abiotrophia adiacens* ATCC 49175, *Abiotrophia defectiva* ATCC 49176, *Bacillus subtilis* ATCC 27370, *Clostridium difficile* ATCC 9689, *Enterococcus avium* ATCC 14025, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus cecorum* ATCC 43198, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus flavescens* ATCC 49996, *Enterococcus gallinarum* ATCC 49573, *Enterococcus solitarius* ATCC 49428, *Escherichia coli* ATCC 11775, *Haemophilus influenzae* ATCC 9006, *Lactobacillus acidophilus* ATCC 4356, *Peptococcus niger* ATCC 27731, *Proteus mirabilis* ATCC 25933, *Staphylococcus aureus* ATCC 43300, *Staphylococcus auricularis* ATCC 33753, *Staphylococcus capitis* ATCC 27840, *Staphylococcus epidemidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus hominis* ATCC 27844, *Staphylococcus lugdunensis* ATCC 43809, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus simulans* ATCC 27848, and *Staphylococcus warneri* ATCC 27836. This primer pair could amplify additional bacterial species; however, there was no amplification for some species, suggesting that the PCR cycling conditions could be optimized or the primers modified. For example, SEQ ID NO. 1227 was designed to amplify a broader range of species.

In addition to other possible primer combinations to amplify the region covering *fusA* and *tuf*, Figure 3 illustrates the positions of amplification primers SEQ ID NOs. 1221-1227 which could be used for universal amplification of *fusA* segments. All of the above mentioned primers (SEQ ID NOs. 1221-1229) could be useful for the universal and/or the specific detection of bacteria.

Moreover, different combinations of primers SEQ ID NOs. 1221-1229, sometimes in combination with *tuf* sequencing primer SEQ ID NO. 697, were used to sequence portions of the *str* operon, including the intergenic region. In this manner, the following sequences were generated: SEQ ID NOs. 1518-1526, 1578-1580, 1786-1821, 1822-1834, 1838-1843, 2184, 2187, 2188, 2214-2249, and 2255-2269.

EXAMPLE 25:

DNA fragment isolation from *Staphylococcus saprophyticus* by arbitrarily primed PCR. DNA sequences of unknown coding potential for the species-specific detection and identification of *Staphylococcus saprophyticus* were obtained by the method of arbitrarily primed PCR (AP-PCR).

AP-PCR is a method which can be used to generate specific DNA probes for microorganisms (Fani *et al.*, 1993, *Molecular Ecology* 2:243-250). A description of the AP-PCR protocol used to isolate a species-specific genomic DNA fragment from *Staphylococcus saprophyticus* follows. Twenty different oligonucleotide primers of 10 nucleotides in length (all included in the AP-PCR kit OPAD (Operon Technologies, Inc., Alameda, CA)) were tested systematically with DNAs from 5 bacterial strains of *Staphylococcus saprophyticus* as well as with bacterial strains of 27 other staphylococcal (non-*S. saprophyticus*) species. For all bacterial species, amplification was performed directly from one μL (0.1 ng/ μL) of purified genomic DNA. The 25 μL PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl_2 , 1.2 μM of only one of the 20 different AP-PCR primers OPAD, 200 μM of each of the four dNTPs, 0.5 U of *Taq* DNA polymerase (Promega Corp., Madison, Wis.) coupled with TaqStartTM antibody (Clontech Laboratories Inc., Palo Alto, CA). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler as follows: 3 min at 96 °C followed by 42 cycles of 1 min at 94 °C for the denaturation step, 1 min at 31 °C for the annealing step and 2 min at 72 °C for the extension step. A final extension step of 7 min at 72 °C followed the 42 cycles to ensure complete extension of PCR products. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis on a 1.5 % agarose gel containing 0.25 $\mu\text{g/ml}$ of ethidium bromide. The size of the amplification products was estimated by comparison with a 50-bp molecular weight ladder.

Amplification patterns specific for *Staphylococcus saprophyticus* were observed with the AP-PCR primer OPAD-16 (sequence: 5'-AACGGGCGTC-3'). Amplification with this primer consistently showed a band corresponding to a

DNA fragment of approximately 380 bp for all *Staphylococcus saprophyticus* strains tested but not for any of the other staphylococcal species tested.

The band corresponding to the 380 bp amplicon, specific and ubiquitous for *S. saprophyticus* based on AP-PCR, was excised from the agarose gel and purified using the QIAquickTM gel extraction kit (QIAGEN Inc.). The gel-purified DNA fragment was cloned into the T/A cloning site of the pCR 2.1TM plasmid vector (Invitrogen Inc.) using T4 DNA ligase (New England BioLabs). Recombinant plasmids were transformed into *E. coli* DH5 α competent cells using standard procedures. All reactions were performed according to the manufacturer's instructions. Plasmid DNA isolation was done by the method of Birnboim and Doly (Nucleic Acid Res., 1979, 7:1513-1523) for small-scale preparations. All plasmid DNA preparations were digested with the EcoRI restriction endonuclease to ensure the presence of the approximately 380 bp AP-PCR insert into the plasmid. Subsequently, a large-scale and highly purified plasmid DNA preparation was performed from two selected clones shown to carry the AP-PCR insert by using the QIAGEN plasmid purification kit (midi format). These large-scale plasmid preparations were used for automated DNA sequencing.

The 380 bp nucleotide sequence was determined for three strains of *S. saprophyticus* (SEQ ID NOs. 74, 1093, and 1198). Both strands of the AP-PCR insert from the two selected clones were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers by using the Applied Biosystems automated DNA sequencer (model 373A) with their PRISMTM Sequenase^{RTM} Terminator Double-stranded DNA Sequencing Kit (Applied Biosystems, Foster City, CA).

Optimal species-specific amplification primers (SEQ ID NOs. 1208 and 1209) have been selected from the sequenced AP-PCR *Staphylococcus saprophyticus* DNA fragments with the help of the primer analysis software OligoTM 5.0 (National BioSciences Inc.). The selected primers were tested in PCR assays to verify their specificity and ubiquity. Data obtained with DNA preparations from reference ATCC strains of 49 gram-positive and 31 gram-negative bacterial

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species, including 28 different staphylococcal species, indicate that the selected primer pairs are specific for *Staphylococcus saprophyticus* since no amplification signal has been observed with DNAs from the other staphylococcal or bacterial species tested. This assay was able to amplify efficiently DNA from all 60 strains of *S. saprophyticus* from various origins tested. The sensitivity level achieved for three *S. saprophyticus* reference ATCC strains was around 6 genome copies.

EXAMPLE 26:

Sequencing of prokaryotic *tuf* gene fragments. The comparison of publicly available *tuf* sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify *tuf* sequences from a wide range of bacterial species. Using primer pair SEQ ID NOs. 664 and 697, it was possible to amplify and determine *tuf* sequences SEQ ID NOs.: 1-73, 75-241, 607-618, 621, 662, 675, 717-736, 868-888, 932, 967-989, 992, 1002, 1572-1575, 1662-1663, 1715-1733, 1835-1837, 1877-1878, 1880-1881, 2183, 2185, 2200, 2201, and 2270-2272.

EXAMPLE 27:

Sequencing of procaryotic *recA* gene fragments. The comparison of publicly available *recA* sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify *recA* sequences from a wide range of bacterial species. Using primer pairs SEQ ID NOs. 921-922 and 1605-1606, it was possible to amplify and determine *recA* sequences SEQ ID NOs.: 990-991, 1003, 1288-1289, 1714, 1756-1763, 1866-1873 and 2202-2212.

EXAMPLE 28:

Specific detection and identification of *Escherichia coli/Shigella* sp. using *tuf* sequences. The analysis of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers (SEQ ID NOs. 1661 and 1665) and of an internal probe (SEQ ID NO. 2168) specific to *Escherichia coli/Shigella* sp. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various *tuf* sequences. The multiple sequence alignment included the *tuf* sequences of *Escherichia coli/Shigella* sp. as well as *tuf* sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from the closely related species, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

The chosen primer pair, oligos SEQ ID NOs. 1661 and 1665, gives an amplification product of 219 bp. Standard PCR was carried out using 0.4 μ M of each primer, 2.5 mM MgCl₂, BSA 0.05 mM, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1 % Triton X-100, dNTPs 0.2 mM (Pharmacia), 0.5 U *Taq* DNA polymerase (Promega) coupled with TaqStartTM antibody (Clontech Laboratories Inc.), 1 μ l of genomic DNA sample in a final volume of 20 μ l using a PTC-200 thermocycler (MJ Research). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μ g/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Escherichia coli* (7

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strains), *Shigella sonnei*, *Shigella flexneri*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella enteritidis*, *Tatumella ptiseos*, *Klebsiella pneumoniae* (2 strains), *Enterobacter aerogenes*, *Citrobacter farmeri*, *Campylobacter jejuni*, *Serratia marcescens*. Amplification was observed only for the *Escherichia coli* and *Shigella* sp. strains listed and *Escherichia fergusonii*. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *E. coli* and three strains of *Shigella* sp. The detection limit for *E. coli* and *Shigella* sp. was 1 to 10 copies of genomic DNA, depending on the strains tested.

EXAMPLE 29:

Specific detection and identification of *Klebsiella pneumoniae* using *atpD* sequences. The analysis of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *K. pneumoniae*. The primer design strategy is similar to the strategy described in Example 28 except that *atpD* sequences were used in the alignment.

Two *K. pneumoniae*-specific primers were selected, (SEQ ID NOs. 1331 and 1332) which give an amplification product of 115 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μ M of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 55°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Klebsiella pneumoniae* (2 strains), *Klebsiella ornitholytica*, *Klebsiella oxytoca* (2 strains), *Klebsiella planticola*, *Klebsiella terrigena*, *Citrobacter freundii*, *Escherichia coli*, *Salmonella cholerasuis typhi*, *Serratia marcescens*, *Enterobacter aerogenes*, *Proteus vulgaris*,

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Kluyvera ascorbata, *Kluyvera georgiana*, *Kluyvera cryocrescens* and *Yersinia enterocolitica*. Amplification was detected for the two *K. pneumoniae* strains, *K. planticola*, *K. terrigena* and the three *Kluyvera* species tested. Analysis of the multiple alignment sequence of the *atpD* gene allowed the design of an internal probe SEQ ID NO. 2167 which can discriminate *Klebsiella pneumoniae* from other *Klebsiella* sp. and *Kluyvera* sp. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *K. pneumoniae*. The detection limit for *K. pneumoniae* was around 10 copies of genomic DNA.

EXAMPLE 30:

Specific detection and identification of *Acinetobacter baumannii* using *atpD* sequences. The analysis of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Acinetobacter baumannii*. The primer design strategy is similar to the strategy described in Example 28.

Two *A. baumannii*-specific primers were selected, SEQ ID NOs. 1690 and 1691, which give an amplification product of 233 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μ M of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Acinetobacter baumannii* (3 strains), *Acinetobacter anitratus*, *Acinetobacter lwoffii*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Psychrobacter phenylpyruvicus*, *Neisseria gonorrhoeae*, *Haemophilus haemoliticus*, *Yersinia enterocolitica*, *Proteus vulgaris*, *Eikenella corrodens*,

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Escherichia coli. Amplification was detected only for *A. baumannii*, *A. anitratus* and *A. lwoffii*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *A. baumannii*. The detection limit for the two *A. baumannii* strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the *atpD* gene allowed the design of a *A. baumannii*-specific internal probe (SEQ ID NO. 2169).

EXAMPLE 31:

Specific detection and identification of *Neisseria gonorrhoeae* using *tuf* sequences.

The analysis of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Neisseria gonorrhoeae*. The primer design strategy is similar to the strategy described in Example 28.

Two *N. gonorrhoeae*-specific primers were selected, SEQ ID NOs. 551 and 552, which give an amplification product of 139 bp. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μ M of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 65°C, followed by terminal extension at 72 °C for 2 minutes.

Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the following bacterial species: *Neisseria gonorrhoeae* (19 strains), *Neisseria meningitidis* (2 strains), *Neisseria lactamica*, *Neisseria flavescens*, *Neisseria animalis*, *Neisseria canis*, *Neisseria cuniculi*, *Neisseria elongata*, *Neisseria mucosa*, *Neisseria polysaccharea*, *Neisseria sicca*, *Neisseria subflava*, *Neisseria weaveri*. Amplification was detected only for *N. gonorrhoeae*, *N. sicca* and *N. polysaccharea*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *N. gonorrhoeae*. The detection limit for the *N.*

gonorrhoeae strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the *tuf* gene allowed the design of an internal probe, SEQ ID NO. 2166, which can discriminate *N. gonorrhoeae* from *N. sicca* and *N. polysaccharea*.

EXAMPLE 32:

Sequencing of bacterial *gyrA* and *parC* gene fragments. Sequencing of bacterial *gyrA* and *parC* fragments. One of the major mechanism of resistance to quinolone in various bacterial species is mediated by target changes (DNA gyrase and/or topoisomerase IV). These enzymes control DNA topology and are vital for chromosome function and replication. Each of these enzymes is a tetramer composed of two subunits: GyrA and GyrB forming A₂B₂ complex in DNA gyrase; and ParC and ParE forming C₂E₂ complex in DNA topoisomerase IV. It has been shown that they are hotspots, called the quinolone-resistance-determining region (QRDR) for mutations within *gyrA* that encodes for the GyrA subunit of DNA gyrase and within *parC* that encodes the *parC* subunit of topoisomerase IV.

In order to generate a database for *gyrA* and *parC* sequences that can be used for design of primers and/or probes for the specific detection of quinolone resistance in various bacterial species, *gyrA* and *parC* DNA fragments selected from public database (GenBank and EMBL) from a variety of bacterial species were used to design oligonucleotide primers.

Using primer pair SEQ ID NOs. 1297 and 1298, it was possible to amplify and determine *gyrA* sequences from *Klebsiella oxytoca* (SEQ ID NO. 1764), *Klebsiella pneumoniae* subsp. *ozaneae* (SEQ ID NO. 1765), *Klebsiella planticola* (SEQ ID NO. 1766), *Klebsiella pneumoniae* (SEQ ID NO. 1767), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1768-1769), *Klebsiella*

pneumoniae subsp. *rhinoscleromatis* (SEQ ID NO. 1770), *Klebsiella terrigena* (SEQ ID NO. 1771), *Kluyvera ascorbata* (SEQ ID NO. 2013), *Kluyvera georgiana* (SEQ ID NO. 2014) and *Escherichia coli* (4 strains) (SEQ ID NOs. 2277-2280). Using primer pair SEQ ID NOs. 1291 and 1292, it was possible to amplify and determine *gyrA* sequences from *Legionella pneumophila* subsp. *pneumophila* (SEQ ID NO. 1772), *Proteus mirabilis* (SEQ ID NO. 1773), *Providencia rettgeri* (SEQ ID NO. 1774), *Proteus vulgaris* (SEQ ID NO. 1775) and *Yersinia enterocolitica* (SEQ ID NO. 1776). Using primer pair SEQ ID NOs. 1340 and 1341, it was possible to amplify and determine *gyrA* sequence from *Staphylococcus aureus* (SEQ ID NO. 1255).

Using primers SEQ ID NOs. 1318 and 1319, it was possible to amplify and determine *parC* sequences from *K. oxytoca* (two strains) (SEQ ID NOs. 1777-1778), *Klebsiella pneumoniae* subsp. *ozaenae* (SEQ ID NO. 1779), *Klebsiella planticola* (SEQ ID NO. 1780), *Klebsiella pneumoniae* (SEQ ID NO. 1781), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1782-1783), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (SEQ ID NO. 1784) and *Klebsiella terrigena* (SEQ ID NO. 1785).

EXAMPLE 33:

Development of a PCR assay for the specific detection and identification of *Staphylococcus aureus* and its quinolone resistance genes *gyrA* and *parC*. The analysis of *gyrA* and *parC* sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from *Staphylococcus aureus*. PCR primer pair SEQ ID NOs. 1340 and 1341 was designed to amplify the *gyrA* sequence of *S. aureus*, whereas PCR primer pair SEQ ID NOs. 1342 and 1343 was designed to amplify *S. aureus parC*. The comparison of *gyrA* and *parC* sequences from *S. aureus* strains with various levels of quinolone resistance

allowed the identification of amino acid substitutions Ser-84 to Leu, Glu-88 to Gly or Lys in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-80 to Phe or Tyr and Ala-116 to Glu in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *S. aureus gyrA* (SEQ ID NO. 1940) and wild-type *S. aureus parC* (SEQ ID NO. 1941) as well as internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 1333-1335) and *parC* mutations identified in quinolone-resistant *S. aureus* (SEQ ID NOs. 1336-1339) were designed.

The *gyrA*- and *parC*-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343) were used in multiplex. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.3, 0.3, 0.6 and 0.6 μ M of each primers, respectively, as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2 %) containing 0.25 μ g/ml of ethidium bromide. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-positive bacteria. The list included the following: *Abiotrophia adiacens*, *Abiotrophia defectiva*, *Bacillus cereus*, *Bacillus mycoides*, *Enterococcus faecalis* (2 strains), *Enterococcus flavescens*, *Gemella morbillorum*, *Lactococcus lactis*, *Listeria innocua*, *Listeria monocytogenes*, *Staphylococcus aureus* (5 strains), *Staphylococcus auricularis*, *Staphylococcus capitis* subsp. *urealyticus*, *Staphylococcus carnosus*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis* (3 strains), *Staphylococcus gallinarum*, *Staphylococcus haemolyticus* (2 strains), *Staphylococcus hominis*, *Staphylococcus hominis* subsp *hominis*, *Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Staphylococcus*

saccharolyticus, *Staphylococcus saprophyticus* (3 strains), *Staphylococcus simulans*, *Staphylococcus warneri*, *Staphylococcus xylosus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*. Strong amplification of both *gyrA* and *parC* genes was only detected for the *S. aureus* strains tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with one quinolone-sensitive and four quinolone-resistant strains of *S. aureus*. The detection limit was 2 to 10 copies of genomic DNA, depending on the strains tested.

Detection of the hybridization with the internal probes was performed as described in Example 7. The internal probes specific to wild-type *gyrA* and *parC* of *S. aureus* and to the *gyrA* and *parC* variants of *S. aureus* were able to recognize two quinolone-resistant and one quinolone-sensitive *S. aureus* strains showing a perfect correlation with the susceptibility to quinolones.

The complete assay for the specific detection of *S. aureus* and its susceptibility to quinolone contains the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7 and the multiplex containing the *S. aureus gyrA*- and *parC*-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. aureus* (SEQ ID NO. 587) described in Example 7 and the internal probes specific to wild-type *S. aureus gyrA* and *parC* (SEQ ID NOs. 1940-1941) and to the *S. aureus gyrA* and *parC* variants (SEQ ID NOs. 1333-1338).

An assay was also developed for the detection of quinolone-resistant *S. aureus* using the SmartCycler (Cepheid). Real-time detection is based on the use of *S. aureus parC*-specific primers (SEQ ID NOs. 1342 and 1343) and the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7. Internal probes were designed for molecular beacon detection of the wild-type *S. aureus parC* (SEQ ID NO.1939), for detection of the Ser-80 to Tyr or

Phe amino acid substitutions in the ParC subunit encoded by *S. aureus parC* (SEQ ID NOs. 1938 and 1955) and for detection of *S. aureus* (SEQ ID NO. 2282) .

EXAMPLE 34:

Development of a PCR assay for the detection and identification of *Klebsiella pneumoniae* and its quinolone resistance genes *gyrA* and *parC*. The analysis of *gyrA* and *parC* sequences from a variety of bacterial species from the public databases and from the database described in Example 32 revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from *K. pneumoniae*. PCR primer pair SEQ ID NOs. 1936 and 1937, or pair SEQ ID NOs. 1937 and 1942, were designed to amplify the *gyrA* sequence of *K. pneumoniae*, whereas PCR primer pair SEQ ID NOs. 1934 and 1935 was designed to amplify *K. pneumoniae parC* sequence. An alternative pair, SEQ ID NOs. 1935 and 1936, can also amplify *K. pneumoniae parC*. The comparison of *gyrA* and *parC* sequences from *K. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-83 to Tyr or Phe and Asp-87 to Gly or Ala and Asp-87 to Asn in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-80 to Ile or Arg and Glu-84 to Gly or Lys in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *K. pneumoniae gyrA* (SEQ ID NO. 1943) and wild-type *K. pneumoniae parC* (SEQ ID NO. 1944) as well as internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 1945-1949) and *parC* mutations identified in quinolone-resistant *K. pneumoniae* (SEQ ID NOs. 1950-1953) were designed.

Two multiplex using the *K. pneumoniae gyrA*- and *parC*-specific primer pairs were used: the first multiplex contained *K. pneumoniae gyrA*-specific primers (SEQ ID

NOs. 1937 and 1942) and *K. pneumoniae parC*-specific primers (SEQ ID NOs. 1934 and 1935) and the second multiplex contained *K. pneumoniae gyrA/parC*-specific primer (SEQ ID NOs. 1936), *K. pneumoniae gyrA*-specific primer (SEQ ID NO. 1937) and *K. pneumoniae parC*-specific primer (SEQ ID NO. 1935). Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using for the first multiplex 0.6, 0.6, 0.4, 0.4 μ M of each primer, respectively, and for the second multiplex 0.8, 0.4, 0.4 μ M of each primer, respectively. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 62 °C, followed by terminal extension at 72 °C for 2 minutes. The specificity of the two multiplex assays with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-negative bacteria. The list included: *Acinetobacter baumannii*, *Citrobacter freundii*, *Eikenella corrodens*, *Enterobacter aerogenes*, *Enterobacter cancerogenes*, *Enterobacter cloacae*, *Escherichia coli* (10 strains), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ornitholytica*, *Klebsiella oxytoca* (2 strains), *Klebsiella planticola*, *Klebsiella terrigena*, *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Kluyvera georgiana*, *Neisseria gonorrhoeae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* subsp. *typhimurium*, *Salmonella enteritidis*, *Serratia liquefaciens*, *Serratia marcescens* and *Yersinia enterocolytica*. For both multiplex, strong amplification of both *gyrA* and *parC* was observed only for the *K. pneumoniae* strain tested. The sensitivity of the two multiplex assays with 40-cycle PCR was verified with one quinolone-sensitive strain of *K. pneumoniae*. The detection limit was around 10 copies of genomic DNA.

The complete assay for the specific detection of *K. pneumoniae* and its susceptibility to quinolone contains the *Klebsiella*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29 and either the multiplex containing the *K.*

pneumoniae gyrA- and *parC*-specific primers (SEQ ID NOs. 1935, 1936, 1937) or the multiplex containing the *K. pneumoniae gyrA*- and *parC*-specific primers (SEQ ID NOs. 1934, 1937, 1939, 1942). Amplification is coupled with post-PCR hybridization with the internal probe specific to *K. pneumoniae* (SEQ ID NO. 2167) described in Example 29 and the internal probes specific to wild-type *K. pneumoniae gyrA* and *parC* (SEQ ID NOs. 1943, 1944) and to the *K. pneumoniae gyrA* and *parC* variants (SEQ ID NOs. 1945-1949 and 1950-1953).

An assay was also developed for the detection of quinolone-resistant *K. pneumoniae* using the SmartCycler (Cepheid). Real-time detection is based on the use of resistant *K. pneumoniae gyrA*-specific primers (SEQ ID NOs. 1936 and 1937) and the *K. pneumoniae*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29. Internal probes were designed for molecular beacon detection of the wild-type *K. pneumoniae gyrA* (SEQ ID NO. 2251), for detection of the Ser-83 to Tyr or Phe and/or Asp-87 to Gly or Asn in the GyrA subunit of DNA gyrase encoded by *gyrA* (SEQ ID NOs. 2250) and for detection of *K. pneumoniae* (SEQ ID NO. 2281).

EXAMPLE 35:

Development of a PCR assay for detection and identification of *S. pneumoniae* and its quinolone resistance genes *gyrA* and *parC*. The analysis of *gyrA* and *parC* sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify the quinolone-resistance-determining region (QRDR) of *gyrA* and *parC* from all *S. pneumoniae* strains. PCR primer pair SEQ ID NOs. 2040 and 2041 was designed to amplify the QRDR of *S. pneumoniae gyrA*, whereas PCR primer pair SEQ ID NOs. 2044 and 2045 was designed to amplify the QRDR of *S. pneumoniae parC*. The comparison of *gyrA* and *parC* sequences from *S. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-81 to Phe or

Tyr in the GyrA subunit of DNA gyrase encoded by *gyrA* and amino acid changes Ser-79 to Phe in the ParC subunit of topoisomerase IV encoded by *parC*. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of each of the *gyrA* (SEQ ID NOs. 2042 and 2043) and *parC* (SEQ ID NO. 2046) mutations identified in quinolone-resistant *S. pneumoniae* were designed.

For all bacterial species, amplification was performed from purified genomic DNA. 1 μ l of genomic DNA at 0.1 ng/ μ L was transferred directly to a 19 μ l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 0.4 μ M (each) of the above primers SEQ ID NOs. 2040, 2041, 2044 and 2045, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase coupled with TaqStartTM antibody. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 58 °C, followed by terminal extension at 72 °C for 2 minutes. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1X PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio ≥ 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria listed in Table 13. Strong amplification of both *gyrA* and *parC* was detected only for the *S. pneumoniae* strains tested. Weak amplification of both *gyrA* and *parC* genes was detected for *Staphylococcus simulans*. The detection limit tested with purified genomic DNA from 5 strains of *S. pneumoniae* was 1 to 10 genome copies. In addition, 5 quinolone-resistant and 2 quinolone-sensitive clinical isolates of *S. pneumoniae* were tested to further validate the developed multiplex PCR coupled with capture probe hybridization assays. There was a perfect correlation between detection of *S. pneumoniae gyrA* and *parC* mutations and the susceptibility to quinolone.

The complete assay for the specific detection of *S. pneumoniae* and its susceptibility to quinolone contains the *S. pneumoniae*-specific primers (SEQ ID NOs. 1179 and 1181) described in Exemple 20 and the multiplex containing the *S. pneumoniae gyrA*-specific and *parC*-specific primer pairs (SEQ ID NOS. 2040 and 2041 and SEQ ID NOs. 2044 and 2045). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) described in Example and the internal probes specific to each of the *S. pneumoniae gyrA* and *parC* variants (SEQ ID NOs. 2042, 2043 and 2046).

EXAMPLE 36:

Detection of extended-spectrum TEM-type β -lactamases in *Escherichia coli*. The analysis of TEM sequences which confer resistance to third-generation cephalosporins and to β -lactamase inhibitors allowed the identification of amino acid substitutions Met-69 to Ile or Leu or Val, Ser-130 to Gly, Arg-164 to Ser or His, Gly-238 to Ser, Glu-240 to Lys and Arg-244 to Ser or Cys or Thr or His or Leu. PCR primers SEQ ID NOs. 1907 and 1908 were designed to amplify TEM sequences. Internal probes for the specific detection of wild-type TEM (SEQ ID NO. 2141) and for each of the amino acid substitutions (SEQ ID NOs. 1909-1926) identified in TEM variants were designed to detect resistance to third-generation

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cephalosporins and to β -lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

For all bacterial species, amplification was performed from purified genomic DNA. One μ l of genomic DNA at 0.1ng/ μ l was transferred directly to a 19 μ l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100, 2.5 mM MgCl₂, 0.4 μ M of the TEM-specific primers SEQ ID NOs. 1907 and 1908, 200 μ M (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase (Promega) coupled with TaqStartTM antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95 °C, 30 seconds at 55 °C and 30 seconds at 72 °C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the TEM-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β -lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). Amplification with the TEM-specific primers was detected only for strains containing TEM.

The sensitivity of the assay with 40-cycle PCR was verified with three *E. coli* strains containing TEM-1 or TEM-10 or TEM-49, one *K. pneumoniae* strain containing TEM-47 and one *P. mirabilis* strain containing TEM-39. The detection

limit was 5 to 100 copies of genomic DNA, depending on the TEM-containing strains tested.

The TEM-specific primers SEQ ID NOs. 1907 and 1908 were used in multiplex with the *Escherichia coli/Shigella sp.*-specific primers SEQ ID NOs. 1661 and 1665 described in Example 28 to allow the complete identification of *Escherichia coli/Shigella sp.* and the susceptibility to β -lactams. PCR amplification with 0.4 μ M of each of the primers and agarose gel analysis of the amplified products was performed as described above.

The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β -lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). The multiplex was highly specific to *Escherichia coli* strains containing TEM.

The complete assay for detection of TEM-type β -lactamases in *E. coli* includes PCR amplification using the multiplex containing the TEM-specific primers (SEQ ID NOs. 1907 and 1908) and the *Escherichia coli/Shigella sp.*-specific primers (SEQ ID NOs. 1661 and 1665) coupled with post PCR-hybridization with the internal probes specific to wild-type TEM (SEQ ID NO. 2141) and to the TEM variants (SEQ ID NOs. 1909-1926).

EXAMPLE 37:

Detection of extended-spectrum SHV-type β -lactamases in *Klebsiella pneumoniae*.

The comparison of SHV sequences, which confer resistance to third-generation

cephalosporins and to β -lactamase inhibitors, allowed the identification of amino acid substitutions Ser-130 to Gly, Asp-179 to Ala or Asn, Gly-238 to Ser, and Glu-240 to Lys. PCR primer pair SEQ ID NOs. 1884 and 1885 was designed to amplify SHV sequences. Internal probes for the specific identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants were designed to detect resistance to third-generation cephalosporins and to β -lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

For all bacterial species, amplification was performed from purified genomic DNA. One μ l of genomic DNA at 0.1ng/ μ l was transferred directly to a 19 μ l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 0.4 μ M of the SHV-specific primers SEQ ID NO. 1884 and 1885, 200 μ M (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U *Taq* polymerase (Promega) coupled with TaqStartTM antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95 °C for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95 °C, 30 seconds at 55 °C and 30 seconds at 72 °C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the SHV-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: two third-generation cephalosporin-resistant *Klebsiella pneumoniae* strains (one with SHV-2a and the other with SHV-12), one third-generation cephalosporin-sensitive *Klebsiella pneumoniae* strain (with SHV-1), two third-generation cephalosporin-resistant *Escherichia coli* strains (one with SHV-8 and the other with SHV-7), and two third-generation cephalosporin-sensitive *Escherichia coli* strains (one with SHV-1

and the other without any SHV). Amplification with the SHV-specific primers was detected only for strains containing SHV.

The sensitivity of the assay with 40-cycle PCR was verified with four strains containing SHV. The detection limit was 10 to 100 copies of genomic DNA, depending on the SHV-containing strains tested.

The amplification was coupled with post-PCR hybridization with the internal probes specific for identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants. The specificity of the probes was verified with six strains containing various SHV enzymes, one *Klebsiella pneumoniae* strain containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one *Klebsiella pneumoniae* strain containing SHV-12, one *Escherichia coli* strain containing SHV-1, one *Escherichia coli* strain containing SHV-7 and one *Escherichia coli* strain containing SHV-8. The probes correctly detected each of the SHV genes and their specific mutations. There was a perfect correlation between the SHV genotype of the strains and the susceptibility to β -lactam antibiotics.

The SHV-specific primers SEQ ID NOs. 1884 and 1885 were used in multiplex with the *K. pneumoniae*-specific primers SEQ ID NOs. 1331 and 1332 described in Example 29 to allow the complete identification of *K. pneumoniae* and the susceptibility to β -lactams. PCR amplification with 0.4 μ M of each of the primers and agarose gel analysis of the amplified products were performed as described above.

The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three *K. pneumoniae* strains containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one

Klebsiella pneumoniae strain containing SHV-12, one *K. rhinoscleromatis* strain containing SHV-1, one *Escherichia coli* strain without SHV. The multiplex was highly specific to *Klebsiella pneumoniae* strain containing SHV.

EXAMPLE 38:

Development of a PCR assay for the detection and identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene *tetM*. The analysis of publicly available *tetM* sequences revealed conserved regions allowing the design of PCR primers specific to *tetM* sequences. The PCR primer pair SEQ ID NOs. 1588 and 1589 was used in multiplex with the *Neisseria gonorrhoeae*-specific primers SEQ ID NOs. 551 and 552 described in Example 31. Sequence alignment analysis of *tetM* sequences revealed regions suitable for the design of an internal probe specific to *tetM* (SEQ ID NO. 2254). PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μ M of each primer pair as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95 °C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95 °C and 30 seconds at 60°C, followed by terminal extension at 72 °C for 2 minutes.

The specificity of the multiplex PCR assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: two tetracycline-resistant *Escherichia coli* strains (one containing the tetracycline-resistant gene *tetB* and the other containing the tetracycline-resistant gene *tetC*), one tetracycline-resistant *Pseudomonas aeruginosa* strain (containing the tetracycline-resistant gene *tetA*), nine tetracycline-resistant *Neisseria gonorrhoeae* strains, two tetracycline-sensitive *Neisseria meningitidis* strains, one tetracycline-sensitive *Neisseria polysaccharea* strain, one tetracycline-sensitive *Neisseria sicca* strain and one tetracycline-sensitive *Neisseria subflava* strain. Amplification with both the *tetM*-specific and *Neisseria gonorrhoeae*-specific primers was detected

only for *N. gonorrhoeae* strains containing *tetM*. There was a weak amplification signal using *Neisseria gonorrhoeae*-specific primers for the following species: *Neisseria sicca*, *Neisseria polysaccharea* and *Neisseria meningitidis*. There was a perfect correlation between the *tetM* genotype and the tetracycline susceptibility pattern of the *Neisseria gonorrhoeae* strains tested. The internal probe specific to *N. gonorrhoeae* SEQ ID NO. 2166 described in Example 31 can discriminate *Neisseria gonorrhoeae* from the other *Neisseria* sp.

The sensitivity of the assay with 40-cycle PCR was verified with two tetracycline resistant strains of *N. gonorrhoeae*. The detection limit was 5 copies of genomic DNA for both strains.

EXAMPLE 39:

Development of a PCR assay for the detection and identification of *Shigella* sp. and their associated trimethoprim resistance gene *dhfrIa*. The analysis of publicly available *dhfrIa* and other *dhfr* sequences revealed regions allowing the design of PCR primers specific to *dhfrIa* sequences. The PCR primer pair (SEQ ID NOs. 1459 and 1460) was used in multiplex with the *Escherichia coli/Shigella* sp.-specific primers SEQ ID NOs. 1661 and 1665 described in Example 28. Sequence alignment analysis of *dhfrIa* sequences revealed regions suitable for the design of an internal probe specific to *dhfrIa* (SEQ ID NO. 2253). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28 with an annealing temperature of 60 °C. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria. The list included the following trimethoprim-sensitive strains, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella enteritidis*, *Tatumella ptyseos*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter farmeri*, *Campylobacter jejuni*, *Serratia marcescens*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, six trimethoprim-resistant *Escherichia coli* strains (containing *dhfrIa* or *dhfrV* or *dhfrVII* or *dhfrXII* or

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dhfrXIII or *dhfrXV*), four trimethoprim-resistant strains containing *dhfrIa* (*Shigella sonnei*, *Shigella flexneri*, *Shigella dysenteriae* and *Escherichia coli*). There was a perfect correlation between the *dhfrIa* genotype and the trimethoprim susceptibility pattern of the *Escherichia coli* and *Shigella* sp. strains tested. The *dhfrIa* primers were specific to the *dhfrIa* gene and did not amplify any of the other trimethoprim-resistant *dhfr* genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of trimethoprim-resistant strains of *Shigella* sp. The detection limit was 5 to 10 genome copies of DNA, depending on the *Shigella* sp. strains tested.

EXAMPLE 40:

Development of a PCR assay for the detection and identification of *Acinetobacter baumannii* and its associated aminoglycoside resistance gene *aph(3')-VIa*. The comparison of publicly available *aph(3')-VIa* sequence revealed regions allowing the design of PCR primers specific to *aph(3')-VIa*. The PCR primer pair (SEQ ID NOs. 1404 and 1405) was used in multiplex with the *Acinetobacter baumannii*-specific primers SEQ ID NOs. 1692 and 1693 described in Example 30. Analysis of the *aph(3')-VIa* sequence revealed region suitable for the design of an internal probe specific to *aph(3')-VIa* (SEQ ID NO. 2252). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria including: two aminoglycoside-resistant *A. baumannii* strains (containing *aph(3')-VIa*), one aminoglycoside-sensitive *A. baumannii* strain, one of each of the following aminoglycoside-resistant bacteria, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene *aacC1*, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene *aacC4*, one *Enterobacter cloacae* strain containing the aminoglycoside-resistant gene *aacC2*, one *Enterococcus faecalis* containing the aminoglycoside-resistant gene *aacA-aphD*, one *Pseudomonas*

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aeruginosa strain containing the aminoglycoside-resistant gene *aac6IIa* and one of each of the following aminoglycoside-sensitive bacterial species, *Acinetobacter anitratus*, *Acinetobacter lwoffii*, *Psychobacter phenylpyruvian*, *Neisseria gonorrhoeae*, *Haemophilus haemolyticus*, *Haemophilus influenzae*, *Yersinia enterocolitica*, *Proteus vulgaris*, *Eikenella corrodens*, *Escherichia coli*. There was a perfect correlation between the *aph(3')-VIa* genotype and the aminoglycoside susceptibility pattern of the *A. baumannii* strains tested. The *aph(3')-VIa*-specific primers were specific to the *aph(3')-VIa* gene and did not amplify any of the other aminoglycoside-resistant genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with two strains of aminoglycoside-resistant strains of *A. baumannii*. The detection limit was 5 genome copies of DNA for both *A. baumannii* strains tested.

EXAMPLE 41:

Specific identification of *Bacteroides fragilis* using *atpD* (V-type) sequences. The comparison of *atpD* (V-type) sequences from a variety of bacterial species allowed the selection of PCR primers for *Bacteroides fragilis*. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various *atpD* sequences from *B. fragilis*, as well as *atpD* sequences from the related species *B. dispar*, bacterial genera and archaea, especially representatives with phylogenetically related *atpD* sequences. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from closely related species *B. dispar*, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

The chosen primer pair, SEQ ID NOs. 2134-2135, produces an amplification product of 231 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc.) using 0.4 μ M of each primers pair as described in Example 28. The

optimal cycling conditions for maximum sensitivity and specificity were as follows: three minutes at 95°C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 60°C, followed by terminal extension at 72°C for 2 minutes.

The format of this assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 2136 for the detection of the *B. fragilis* amplicon.

EXAMPLE 42:

Evidence for horizontal gene transfer in the evolution of the elongation factor Tu in Enterococci.

ABSTRACT

The elongation factor Tu, encoded by *tuf* genes, is a GTP binding protein that plays a central role in protein synthesis. One to three *tuf* genes per genome are present depending on the bacterial species. Most low G+C gram-positive bacteria carry only one *tuf* gene. We have designed degenerate PCR primers derived from consensus sequences of the *tuf* gene to amplify partial *tuf* sequences from 17 enterococcal species and other phylogenetically related species. The amplified DNA fragments were sequenced either by direct sequencing or by sequencing cloned inserts containing putative amplicons. Two different *tuf* genes (*tufA* and *tufB*) were found in 11 enterococcal species, including *Enterococcus avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*. For the other six enterococcal species (*E. cecorum*, *E. columbae*, *E. faecalis*, *E. sulfureus*, *E.*

saccharolyticus, and *E. solitarius*), only the *tufA* gene was present. Based on 16S rRNA gene sequence analysis, the 11 species having two *tuf* genes all share a common ancestor, while the six species having only one copy diverged from the enterococcal lineage before that common ancestor. The presence of one or two copies of the *tuf* gene in enterococci was confirmed by Southern hybridization. Phylogenetic analysis of *tuf* sequences demonstrated that the enterococcal *tufA* gene branches with the *Bacillus*, *Listeria* and *Staphylococcus* genera, while the enterococcal *tufB* gene clusters with the genera *Streptococcus* and *Lactococcus*. Primary structure analysis showed that four amino acid residues within the sequenced regions are conserved and unique to the enterococcal *tufB* genes and the *tuf* genes of streptococci and *L. lactis*. The data suggest that an ancestral streptococcus or a streptococcus-related species may have horizontally transferred a *tuf* gene to the common ancestor of the 11 enterococcal species which now carry two *tuf* genes.

INTRODUCTION

The elongation factor Tu (EF-Tu) is a GTP binding protein playing a central role in protein synthesis. It mediates the recognition and transport of aminoacyl-tRNAs and their positioning to the A-site of the ribosome. The highly conserved function and ubiquitous distribution render the elongation factor a valuable phylogenetic marker among eubacteria and even throughout the archaeobacterial and eukaryotic kingdoms. The *tuf* genes encoding elongation factor Tu are present in various copy numbers per bacterial genome. Most gram-negative bacteria contain two *tuf* genes. As found in *Escherichia coli*, the two genes, while being almost identical in sequence, are located in different parts of the bacterial chromosome. However, recently completed microbial genomes revealed that only one *tuf* gene is found in *Helicobacter pylori* as well as in some obligate parasitic bacteria, such as *Borrelia burgdorferi*, *Rickettsia prowazekii*, and *Treponema pallidum*, and in some cyanobacteria. In most gram-positive bacteria studied so far, only one *tuf* gene was found. However, Southern hybridization showed that there are two *tuf* genes in

some clostridia as well as in *Streptomyces coelicolor* and *S. lividans*. Up to three *tuf*-like genes have been identified in *S. ramocissimus*.

Although massive prokaryotic gene transfer is suggested to be one of the factors responsible for the evolution of bacterial genomes, the genes encoding components of the translation machinery are thought to be highly conserved and difficult to be transferred horizontally due to the complexity of their interactions. However, a few recent studies demonstrated evidence that horizontal gene transfer has also occurred in the evolution of some genes coding for the translation apparatus, namely, 16S rRNA and some aminoacyl-tRNA synthetases. No further data suggest that such a mechanism is involved in the evolution of the elongation factors. Previous studies concluded that the two copies of *tuf* genes in the genomes of some bacteria resulted from an ancient event of gene duplication. Moreover, a study of the *tuf* gene in *R. prowazekii* suggested that intrachromosomal recombination has taken place in the evolution of the genome of this organism.

To date, little is known about the *tuf* genes of enterococcal species. In this study, we analyzed partial sequences of *tuf* genes in 17 enterococcal species, namely, *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. columbae*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, *E. raffinosus*, *E. saccharolyticus*, *E. solitarius*, and *E. sulfureus*. We report here the presence of two divergent copies of *tuf* genes in 11 of these enterococcal species. The 6 other species carried a single *tuf* gene. The evolutionary implications are discussed.

MATERIALS AND METHODS

Bacterial strains. Seventeen enterococcal strains and other gram-positive bacterial strains obtained from the American Type Culture Collection (ATCC, Manassas, Va.) were used in this study (Table 16). All strains were grown on sheep blood agar or in brain-heart infusion broth prior to DNA isolation.

DNA isolation. Bacterial DNAs were prepared using the G NOME DNA extraction kit (Bio101, Vista, Calif.) as previously described.

Sequencing of putative *tuf* genes. In order to obtain the *tuf* gene sequences of enterococci and other gram-positive bacteria, two sequencing approaches were used: 1) sequencing of cloned PCR products and 2) direct sequencing of PCR products. A pair of degenerate primers (SEQ ID NOs. 664 and 697) were used to amplify an 886-bp portion of the *tuf* genes from enterococcal species and other gram-positive bacteria as previously described. For *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*, and *E. raffinosus*, the amplicons were cloned using the Original TA cloning kit (Invitrogen, Carlsbad, Calif.) as previously described. Five clones for each species were selected for sequencing. For *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* as well as the other gram-positive bacteria, the sequences of the 886-bp amplicons were obtained by direct sequencing. Based on the results obtained from the earlier rounds of sequencing, two pairs of primers were designed for obtaining the partial *tuf* sequences from the other enterococcal species by direct sequencing. One pair of primers (SEQ ID NOs. 543 and 660) were used to amplify the enterococcal *tuf* gene fragments from *E. columbae*, *E. malodoratus*, and *E. sulfureus*. Another pair of primers (SEQ ID NOs. 664 and 661) were used to amplify the second *tuf* gene fragments from *E. avium*, *E. malodoratus*, and *E. pseudoavium*.

Prior to direct sequencing, PCR products were electrophoresed on 1% agarose gel at 120V for 2 hours. The gel was then stained with 0.02% methylene blue for 30 minutes and washed twice with autoclaved distilled water for 15 minutes. The gel slices containing PCR products of the expected sizes were cut out and purified with the QIAquick gel extraction kit (QIAGEN Inc., Mississauga, Ontario, Canada) according to the manufacturer's instructions. PCR mixtures for sequencing were prepared as described previously. DNA sequencing was carried out with the Big Dye™ Terminator Ready Reaction cycle sequencing kit using a 377 DNA sequencer (PE Applied Biosystems, Foster City, Calif.). Both strands of the

amplified DNA were sequenced. The sequence data were verified using the SequencerTM 3.0 software (Gene Codes Corp., Ann Arbor, Mich.).

Sequence analysis and phylogenetic study. Nucleotide sequences of the *tuf* genes and their respective flanking regions for *E. faecalis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, were retrieved from the TIGR microbial genome database and *S. pyogenes* from the University of Oklahoma database. DNA sequences and deduced protein sequences obtained in this study were compared with those in all publicly available databases using the BLAST and FASTA programs. Unless specified, sequence analysis was conducted with the programs from GCG package (Version 10; Genetics Computer Group, Madison, Wisc.). Sequence alignment of the *tuf* genes from 74 species representing all three kingdoms of life (Tables 16 and 17) were carried out by use of Pileup and corrected upon visual analysis. The N- and C-termini extremities of the sequences were trimmed to yield a common block of 201 amino acids sequences and equivocal residues were removed. Phylogenetic analysis was performed with the aid of PAUP 4.0b4 written by Dr. David L. Swofford (Sinauer Associates, Inc., Publishers, Sunderland, Mass.). The distance matrix and maximum parsimony were used to generate phylogenetic trees and bootstrap resampling procedures were performed using 500 and 100 replications in each analysis, respectively.

Protein structure analysis. The crystal structures of (i) *Thermus aquaticus* EF-Tu in complex with Phe-tRNA^{Phe} and a GTP analog and (ii) *E. coli* EF-Tu in complex with GDP served as templates for constructing the equivalent models for enterococcal EF-Tu. Homology modeling of protein structure was performed using the SWISS-MODEL server and inspected using the SWISS-PDB viewer version 3.1.

Southern hybridization. In a previous study, we amplified and cloned an 803-bp PCR product of the *tuf* gene fragment from *E. faecium*. Two divergent sequences of the inserts, which we assumed to be *tufA* and *tufB* genes, were obtained. The recombinant plasmid carrying either *tufA* or *tufB* sequence was used to generate two probes labeled with Digoxigenin (DIG)-11-dUTP by PCR

incorporation following the instructions of the manufacturer (Boehringer Mannheim, Laval, Québec, Canada). Enterococcal genomic DNA samples (1-2 µg) were digested to completion with restriction endonucleases *Bgl*II and *Xba*I as recommended by the supplier (Amersham Pharmacia Biotech, Mississauga, Ontario, Canada). These restriction enzymes were chosen because no restriction sites were observed within the amplified *tuf* gene fragments of most enterococci. Southern blotting and filter hybridization were performed using positively charged nylon membranes (Boehringer Mannheim) and QuikHyb hybridization solution (Stratagene Cloning Systems, La Jolla, Calif.) according to the manufacturers' instructions with modifications. Twenty µl of each digestion were electrophoresed for 2 h at 120V on a 0.8% agarose gel. The DNA fragments were denatured with 0.5 M NaOH and transferred by Southern blotting onto a positively charged nylon membrane (Boehringer Mannheim). The filters were pre-hybridized for 15 min and then hybridized for 2 h in the QuikHyb solution at 68°C with either DIG-labeled probe. Posthybridization washings were performed twice with 0.5x SSC, 1% SDS at room temperature for 15 min and twice in the same solution at 60°C for 15 min. Detection of bound probes was achieved using disodium 3- (4-methoxyspiro (1,2-dioxetane-3,2'- (5'-chloro) tricyclo(3,3.1.1^{3,7}) decan)-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim) as specified by the manufacturer.

GenBank submission. The GenBank accession numbers for partial *tuf* gene sequences generated in this study are given in Table 16.

RESULTS

Sequencing and nucleotide sequence analysis. In this study, all gram-positive bacteria other than enterococci yielded a single *tuf* sequence of 886 bp using primers SEQ ID NOs. 664 and 697 (Table 16). Each of four enterococcal species including *E. cecorum*, *E. faecalis*, *E. saccharolyticus*, and *E. solitarius* also yielded one 886-bp *tuf* sequence. On the other hand, for *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. pseudoavium*,

and *E. raffinosus*, direct sequencing of the 886-bp fragments revealed overlapping peaks according to their sequence chromatograms, suggesting the presence of additional copies of the *tuf* gene. Therefore, the *tuf* gene fragments of these 10 species were cloned first and then sequenced. Sequencing data revealed that two different types of *tuf* sequences (*tufA* and *tufB*) are found in eight of these species including *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, and *E. raffinosus*. Five clones from *E. avium* and *E. pseudoavium* yielded only a single *tuf* sequence. These new sequence data allowed the design of new primers specific for the enterococcal *tufA* or *tufB* sequences. Primers SEQ ID NOs. 543 and 660 were designed to amplify only enterococcal *tufA* sequences and a 694-bp fragment was amplified from all 17 enterococcal species. The 694-bp sequences of *tufA* genes from *E. columbae*, *E. malodoratus*, and *E. sulfureus* were obtained by direct sequencing using these primers. Primers SEQ ID NOs. 664 and 661 were designed for the amplification of 730-bp portion of *tufB* genes and yielded the expected fragments from 11 enterococcal species, including *E. malodoratus* and the 10 enterococcal species in which heterogeneous *tuf* sequences were initially found. The sequences of the *tufB* fragments for *E. avium*, *E. malodoratus* and *E. pseudoavium* were determined by direct sequencing using the primers SEQ ID NOs. 664 and 661. Overall, *tufA* gene fragments were obtained from all 17 enterococcal species but *tufB* gene fragments were obtained with only 11 enterococcal species (Table 16).

The identities between *tufA* and *tufB* for each enterococcal species were 68-79% at the nucleotide level and 81 to 89% at the amino acid level. The *tufA* gene is highly conserved among all enterococcal species with identities varying from 87% to 99% for DNA and 93% to 99% for amino acid sequences, while the identities among *tufB* genes of enterococci varies from 77% to 92% for DNA and 91% to 99% for amino acid sequences, indicating their different origins and evolution (Table 18). Since *E. solitarius* has been transferred to the genus *Tetragenococcus*, which is also a low G+C gram-positive bacterium, our sequence comparison did not include this species as an enterococcus. G+C content of enterococcal *tufA*

sequences ranged from 40.8% to 43.1%, while that of enterococcal *tufB* sequences varied from 37.8% to 46.3%. Based on amino acid sequence comparison, the enterococcal *tufA* gene products share higher identities with those of *Abiotrophia adiacens*, *Bacillus subtilis*, *Listeria monocytogenes*, *S. aureus*, and *S. epidermidis*. On the other hand, the enterococcal *tufB* gene products share higher percentages of amino acid identity with the *tuf* genes of *S. pneumoniae*, *S. pyogenes* and *Lactococcus lactis* (Table 18).

In order to elucidate whether the two enterococcal *tuf* sequences encode genuine EF-Tu, the deduced amino acid sequences of both genes were aligned with other EF-Tu sequences available in SWISSPROT (Release 38). Sequence alignment demonstrated that both gene products are highly conserved and carry all conserved residues present in this portion of prokaryotic EF-Tu (Figure 4). Therefore, it appears that both gene products could fulfill the function of EF-Tu. The partial *tuf* gene sequences encode the portion of EF-Tu from residues 117 to 317, numbered as in *E. coli*. This portion makes up of the last four α -helices and two β -strands of domain I, the entire domain II and the N-terminal part of domain III on the basis of the determined structures of *E. coli* EF-Tu.

Based on the deduced amino acid sequences, the enterococcal *tufB* genes have unique conserved residues Lys129, Leu140, Ser230, and Asp234 (*E. coli* numbering) that are also conserved in streptococci and *L. lactis*, but not in the other bacteria (Figure 4). All these residues are located in loops except for Ser230. In other bacteria the residue Ser230 is substituted for highly conserved Thr, which is the 5th residue of the third β -strand of domain II. This region is partially responsible for the interaction between the EF-Tu and aminoacyl-tRNA by the formation of a deep pocket for any of the 20 naturally occurring amino acids. According to our three-dimensional model (data not illustrated), the substitution Thr230→Ser in domain II of EF-Tu may have little impact on the capability of the pocket to accommodate any amino acid. However, the high conservation of Thr230 comparing to the unique Ser substitution found only in streptococci and 11 enterococci could suggest a subtle functional role for this residue.

The *tuf* gene sequences obtained for *E. faecalis*, *S. aureus*, *S. pneumoniae* and *S. pyogenes* were compared with their respective incomplete genome sequence. Contigs with more than 99% identity were identified. Analysis of the *E. faecalis* genome data revealed that the single *E. faecalis tuf* gene is located within an *str* operon where *tuf* is preceded by *fus* that encodes the elongation factor G. This *str* operon is present in *S. aureus* and *B. subtilis* but not in the two streptococcal genomes examined. The 700-bp or so sequence upstream the *S. pneumoniae tuf* gene has no homology with any known gene sequences. In *S. pyogenes*, the gene upstream of *tuf* is similar to a cell division gene, *ftsW*, suggesting that the *tuf* genes in streptococci are not arranged in a *str* operon.

Phylogenetic analysis. Phylogenetic analysis of the *tuf* amino acid sequences with representatives of eubacteria, archeabacteria, and eukaryotes using neighbor-joining and maximum parsimony methods showed three major clusters representing the three kingdoms of life. Both methods gave similar topologies consistent with the rRNA gene data (data not shown). Within the bacterial clade, the tree is polyphyletic but *tufA* genes from all enterococcal species always clustered with those from other low G+C gram-positive bacteria (except for streptococci and lactococci), while the *tufB* genes of the 11 enterococcal species form a distinct cluster with streptococci and *L. lactis* (Figure 5). Duplicated genes from the same organism do not cluster together, thereby not suggesting evolution by recent gene duplication.

Southern hybridization. Southern hybridization of *Bgl*III/*Xba*I digested genomic DNA from 12 enterococcal species tested with the *tufA* probe (DIG-labeled *tufA* fragment from *E. faecium*) yielded two bands of different sizes in 9 species, which also carried two divergent *tuf* sequences according to their sequencing data. For *E. faecalis* and *E. solitarius*, a single band was observed indicating that one *tuf* gene is present (Figure 6). A single band was also found when digested genomic DNA from *S. aureus*, *S. pneumoniae*, and *S. pyogenes* were hybridized with the *tufA* probe (data not shown). For *E. faecium*, the presence of three bands can be explained by the existence of a *Xba*I restriction site in the

middle of the *tufA* sequence, which was confirmed by sequencing data. Hybridization with the *tufB* probe (DIG-labeled *tufB* fragment of *E. faecium*) showed a banding profile similar to the one obtained with the *tufA* probe (data not shown).

DISCUSSION

In this study, we have shown that two divergent copies of genes encoding the elongation factor Tu are present in some enterococcal species. Sequence data revealed that both genes are highly conserved at the amino acid level. One copy (*tufA*) is present in all enterococcal species, while the other (*tufB*) is present only in 11 of the 17 enterococcal species studied. Based on 16S rRNA sequence analysis, these 11 species are members of three different enterococcal subgroups (*E. avium*, *E. faecium*, and *E. gallinarum* species groups) and a distinct species (*E. dispar*). Moreover, 16S rDNA phylogeny suggests that these 11 species possessing 2 *tuf* genes all share a common ancestor before they further evolved to become the modern species. Since the six other species having only one copy diverged from the enterococcal lineage before that common ancestor, it appears that the presence of one *tuf* gene in these six species is not attributable to gene loss.

Two clusters of low G+C gram-positive bacteria were observed in the phylogenetic tree of the *tuf* genes: one contains a majority of low G+C gram-positive bacteria and the other contains lactococci and streptococci. This is similar to the finding on the basis of phylogenetic analysis of the 16S rRNA gene and the *hrcA* gene coding for a unique heat-shock regulatory protein. The enterococcal *tufA* genes branched with most of the low G+C gram-positive bacteria, suggesting that they originated from a common ancestor. On the other hand, the enterococcal *tufB* genes branched with the genera *Streptococcus* and *Lactococcus* that form a distinct lineage separated from other low G+C gram-positive bacteria (Figure 5). The finding that these EF-Tu proteins share some conserved amino acid residues unique to this branch also supports the idea that they may share a common ancestor. Although these conserved residues might result from convergent

evolution upon a specialized function, such convergence at the sequence level, even for a few residues, seems to be rare, making it an unlikely event. Moreover, no currently known selective pressure, if any, would account for keeping one versus two *tuf* genes in bacteria. The G+C contents of enterococcal *tufA* and *tufB* sequences are similar, indicating that they both originated from low G+C gram-positive bacteria, in accordance with the phylogenetic analysis.

The *tuf* genes are present in various copy numbers in different bacteria. Furthermore, the two *tuf* genes are normally associated with characteristic flanking genes. The two *tuf* gene copies commonly encountered within gram-negative bacteria are part of the bacterial *str* operon and tRNA-*tufB* operon, respectively. The arrangement of *tufA* in the *str* operon was also found in a variety of bacteria, including *Thermotoga maritima*, the most ancient bacteria sequenced so far, *Aquifex aeolicus*, cyanobacteria, *Bacillus* sp., *Micrococcus luteus*, *Mycobacterium tuberculosis*, and *Streptomyces* sp. Furthermore, the tRNA-*tufB* operon has also been identified in *Aquifex aeolicus*, *Thermus thermophilus*, and *Chlamydia trachomatis*. The two widespread *tuf* gene arrangements argue in favor of their ancient origins. It is noteworthy that most obligate intracellular parasites, such as *Mycoplasma* sp., *R. prowazekii*, *B. burgdorferi*, and *T. pallidum*, contain only one *tuf* gene. Their flanking sequences are distinct from the two conserved patterns as a result of selection for effective propagation by an extensive reduction in genome size by intragenomic recombination and rearrangement.

Most gram-positive bacteria with low G+C content sequenced to date contain only a single copy of the *tuf* gene as a part of the *str* operon. This is the case for *B. subtilis*, *S. aureus* and *E. faecalis*. PCR amplification using a primer targeting a conserved region of the *fus* gene and the *tufA*-specific primer SEQ ID NO. 660, but not the *tufB*-specific primer SEQ ID NO. 661, yielded the expected amplicons for all 17 enterococcal species tested, indicating the presence of the *fus-tuf* organization in all enterococci (data not shown). However, in the genomes of *S. pneumoniae* and *S. pyogenes*, the sequences flanking the *tuf* genes varies although the *tuf* gene itself remains highly conserved. The enterococcal *tufB* genes are

clustered with streptococci, but at present we do not have enough data to identify the genes flanking the enterococcal *tufB* genes. Furthermore, the functional role of the enterococcal *tufB* genes remains unknown. One can only postulate that the two divergent gene copies are expressed under different conditions.

The amino acid sequence identities between the enterococcal *tufA* and *tufB* genes are lower than either i) those between the enterococcal *tufA* and the *tuf* genes from other low G+C gram-positive bacteria (streptococci and lactococci excluded) or ii) those between the enterococcal *tufB* and streptococcal and lactococcal *tuf* genes. These findings suggest that the enterococcal *tufA* genes share a common ancestor with other low G+C gram-positive bacteria via the simple scheme of vertical evolution, while the enterococcal *tufB* genes are more closely related to those of streptococci and lactococci. The facts that some enterococci possess an additional *tuf* gene and that the single streptococcal *tuf* gene is not clustered with other low G+C gram-positive bacteria cannot be explained by the mechanism of gene duplication or intrachromosomal recombination. According to sequence and phylogenetic analysis, we propose that the presence of the additional copy of the *tuf* genes in 11 enterococcal species is due to horizontal gene transfer. The common ancestor of the 11 enterococcal species now carrying *tufB* genes acquired a *tuf* gene from an ancestral streptococcus or a streptococcus-related species during enterococcal evolution through gene transfer before the diversification of modern enterococci. Further study of the flanking regions of the gene may provide more clues for the origin and function of this gene in enterococci.

Recent studies of genes and genomes have demonstrated that considerable horizontal transfer occurred in the evolution of aminoacyl-tRNA synthetases in all three kingdoms of life. The heterogeneity of 16S rRNA is also attributable to horizontal gene transfer in some bacteria, such as *Streptomyces*, *Thermomonospora chromogena* and *Mycobacterium celatum*. In this study, we provide the first example in support of a likely horizontal transfer of the *tuf* gene encoding the elongation factor Tu. This may be an exception since stringent functional constraints do not allow for frequent horizontal transfer of the *tuf* gene as with

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other genes. However, enterococcal *tuf* genes should not be the only such exception as we have noticed that the phylogeny of *Streptomyces tuf* genes is equally or more complex than that of enterococci. For example, the three *tuf*-like genes in a high G+C gram-positive bacterium, *S. ramocissimus*, branched with the *tuf* genes of phylogenetically divergent groups of bacteria (Figure 5). Another example may be the *tuf* genes in clostridia, which represent a phylogenetically very broad range of organisms and form a plethora of lines and groups of various complexities and depths. Four species belonging to three different clusters within the genus *Clostridium* have been shown by Southern hybridization to carry two copies of the *tuf* gene. Further sequence data and phylogenetic analysis may help interpreting the evolution of the elongation factor Tu in these gram-positive bacteria. Since the *tuf* genes and 16S rRNA genes are often used for phylogenetic study, the existence of duplicate genes originating from horizontal gene transfer may alter the phylogeny of microorganisms when the laterally acquired copy of the gene is used for such analysis. Hence, caution should be taken in interpreting phylogenetic data. In addition, the two *tuf* genes in enterococci have evolved separately and are distantly related to each other phylogenetically. The enterococcal *tufB* genes are less conserved and unique to the 11 enterococcal species only. We previously demonstrated that the enterococcal *tufA* genes could serve as a target to develop a DNA-based assay for identification of enterococci. The enterococcal *tufB* genes would also be useful in identification of these 11 enterococcal species.

EXAMPLE 43:

Elongation Factor Tu (*tuf*) and the F-ATPase beta-subunit (*atpD*) as phylogenetic tools for species of the family *Enterobacteriaceae*.

SUMMARY

The phylogeny of enterobacterial species commonly found in clinical samples was analyzed by comparing partial sequences of their elongation factor Tu (*tuf*) genes and their F-ATPase beta-subunit (*atpD*) genes. A 884-bp fragment for *tuf* and a 884- or 871-bp fragment for *atpD* were sequenced for 88 strains of 72 species from 25 enterobacterial genera. The *atpD* sequence analysis revealed a specific indel to *Pantoea* and *Tatumella* species showing for the first time a tight phylogenetic affiliation between these two genera. Comprehensive *tuf* and *atpD* phylogenetic trees were constructed and are in agreement with each other. Monophyletic genera are *Yersinia*, *Pantoea*, *Edwardsiella*, *Cedecea*, *Salmonella*, *Serratia*, *Proteus*, and *Providencia*. Analogous trees were obtained based on available 16S rDNA sequences from databases. *tuf* and *atpD* phylogenies are in agreement with the 16S rDNA analysis despite the smaller resolution power for the latter. In fact, distance comparisons revealed that *tuf* and *atpD* genes provide a better resolution for pairs of species belonging to the family *Enterobacteriaceae*. However, 16S rDNA distances are better resolved for pairs of species belonging to different families. In conclusion, *tuf* and *atpD* conserved genes are sufficiently divergent to discriminate different species inside the family *Enterobacteriaceae* and offer potential for the development of diagnostic tests based on DNA to identify enterobacterial species.

INTRODUCTION

Members of the family *Enterobacteriaceae* are facultatively anaerobic gram-negative rods, catalase-positive and oxydase-positive (Brenner, 1984). They are found in soil, water, plants, and in animals from insects to man. Many enterobacteria are opportunistic pathogens. In fact, members of this family are responsible for about 50 % of nosocomial infections in the United States (Brenner, 1984). Therefore, this family is of considerable clinical importance.

Major classification studies on the family *Enterobacteriaceae* are based on phenotypic traits (Brenner *et al.*, 1999; Brenner *et al.*, 1980; Dickey & Zumoff,

1988; Farmer III *et al.*, 1980; Farmer III *et al.*, 1985b; Farmer III *et al.*, 1985a) such as biochemical reactions and physiological characteristics. However, phenotypically distinct strains may be closely related by genotypic criteria and may belong to the same genospecies (Bercovier *et al.*, 1980; Hartl & Dykhuizen, 1984). Also, phenotypically close strains (biogroups) may belong to different genospecies, like *Klebsiella pneumoniae* and *Enterobacter aerogenes* (Brenner, 1984) for example. Consequently, identification and classification of certain species may be ambiguous with techniques based on phenotypic tests (Janda *et al.*, 1999; Kitch *et al.*, 1994; Sharma *et al.*, 1990).

More advances in the classification of members of the family *Enterobacteriaceae* have come from DNA-DNA hybridization studies (Brenner *et al.*, 1993; Brenner *et al.*, 1986; Brenner, *et al.*, 1980; Farmer III, *et al.*, 1980; Farmer III, *et al.*, 1985b; Izard *et al.*, 1981; Steigerwalt *et al.*, 1976). Furthermore, the phylogenetic significance of bacterial classification based on 16S rDNA sequences has been recognized by many workers (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). However, members of the family *Enterobacteriaceae* have not been subjected to extensive phylogenetic analysis of 16S rDNA (Sproer *et al.*, 1999). In fact, this molecule was not thought to solve taxonomic problems concerning closely related species because of its very high degree of conservation (Brenner, 1992; Sproer, *et al.*, 1999). Another drawback of the 16S rDNA gene is that it is found in several copies within the genome (seven in *Escherichia coli* and *Salmonella typhimurium*) (Hill & Harnish, 1981). Due to sequence divergence between the gene copies, direct sequencing of PCR products is often not suitable to achieve a representative sequence (Cilia *et al.*, 1996; Hill & Harnish, 1981). Other genes such as *gap* and *ompA* (Lawrence *et al.*, 1991), *rpoB* (Mollet *et al.*, 1997), and *infB* (Hedegaard *et al.*, 1999) were used to resolve the phylogeny of enterobacteria. However, none of these studies covered an extensive number of species.

tuf and *atpD* are the genes encoding the elongation factor Tu (EF-Tu) and the F-ATPase beta-subunit, respectively. EF-Tu is involved in peptide chain formation (Ludwig *et al.*, 1990). The two copies of the *tuf* gene (*tufA* and *tufB*) found in enterobacteria (Sela *et al.*, 1989) share high identity level (99 %) in *Salmonella typhimurium* and in *E. coli*. The recombination phenomenon could explain sequence homogenization between the two copies (Abdulkarim & Hughes, 1996; Grunberg-Manago, 1996). F-ATPase is present on the plasma membranes of eubacteria (Nelson & Taiz, 1989). It functions mainly in ATP synthesis (Nelson & Taiz, 1989) and the beta-subunit contains the catalytic site of the enzyme. EF-Tu and F-ATPase are highly conserved throughout evolution and shows functional constancy (Amann *et al.*, 1988; Ludwig, *et al.*, 1990). Recently, phylogenies based on protein sequences from EF-Tu and F-ATPase beta-subunit showed good agreement with each other and with the rDNA data (Ludwig *et al.*, 1993).

We elected to sequence 884-bp fragments of *tuf* and *atpD* from 88 clinically relevant enterobacterial strains representing 72 species from 25 genera. These sequences were used to create phylogenetic trees that were compared with 16S rDNA trees. These trees revealed good agreement with each others and demonstrated the high resolution of *tuf* and *atpD* phylogenies at the species level.

MATERIALS AND METHODS

Bacterial strains and genomic material. All bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) or the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). These enterobacteria can all be recovered from clinical specimens, but not all are pathogens. Whenever possible, we choose type strains. Identification of all strains was confirmed by classical biochemical tests using the automated system MicroScan WalkAway-96 system equipped with a Negative BP Combo Panel Type 15 (Dade Behring Canada). Genomic DNA was purified using the G NOME

DNA kit (Bio 101). Genomic DNA from *Yersinia pestis* was kindly provided by Dr. Robert R. Brubaker. Strains used in this study and their descriptions are shown in Table 19.

PCR primers. The eubacterial *tuf* and *atpD* gene sequences available from public databases were analyzed using the GCG package (version 8.0) (Genetics Computer Group). Based on multiple sequence alignments, two highly conserved regions were chosen for each genes, and PCR primers were derived from these regions with the help of Oligo primer analysis software (version 5.0) (National Biosciences). A second 5' primer was design to amplify the gene *atpD* for few enterobacteria difficult to amplify with the first primer set. When required, the primers contained inosines or degeneracies to account for variable positions. Oligonucleotide primers were synthesized with a model 394 DNA/RNA synthesizer (PE Applied Biosystems). PCR primers used in this study are listed in Table 20.

DNA sequencing. An 884-bp portion of the *tuf* gene and an 884-bp portion (or alternatively an 871-bp portion for a few enterobacterial strains) of the *atpD* gene were sequenced for all enterobacteria listed in the first strain column of Table 19. Amplification was performed with 4 ng of genomic DNA. The 40- μ l PCR mixtures used to generate PCR products for sequencing contained 1.0 μ M each primer, 200 μ M each deoxyribonucleoside triphosphate (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0 at 25 °C), 50 mM KCl, 0.1 % (w/v) Triton X-100, 2.5 mM MgCl₂, 0.05 mM BSA, 0.3 U of *Taq* DNA polymerase (Promega) coupled with TaqStartTM antibody (Clontech Laboratories). The TaqStartTM neutralizing monoclonal antibody for *Taq* DNA polymerase was added to all PCR mixtures to enhance efficiency of amplification (Kellogg *et al.*, 1994). The PCR mixtures were subjected to thermal cycling (3 min at 95 °C and then 35 cycles of 1 min at 95 °C, 1 min at 55 °C for *tuf* or 50 °C for *atpD*, and 1 min at 72 °C, with a 7-min final extension at 72 °C) using a PTC-200 DNA Engine thermocycler (MJ Research).

PCR products having the predicted sizes were recovered from an agarose gel stained for 15 min with 0.02 % of methylene blue followed by washing in sterile distilled water for 15 min twice (Flores *et al.*, 1992). Subsequently, PCR products having the predicted sizes were recovered from gels using the QIAquick gel extraction kit (QIAGEN).

Both strands of the purified amplicons were sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) on an automated DNA sequencer (Model 377). Amplicons from two independent PCR amplifications were sequenced for each strain to ensure the absence of sequencing errors attributable to nucleotide misincorporations by the *Taq* DNA polymerase. Sequence assembly was performed with the aid of Sequencher 3.0 software (Gene Codes).

Phylogenetic analysis. Multiple sequence alignments were performed using PileUp from the GCG package (Version 10.0) (Genetics Computer Group) and checked by eye with the editor SeqLab to edit sequences if necessary and to note which regions were to be excluded for phylogenetic analysis. *Vibrio cholerae* and *Shewanella putrefaciens* were used as outgroups. Bootstrap subsets (750 sets) and phylogenetic trees were generated with the Neighbor Joining algorithm from Dr. David Swofford's PAUP (Phylogenetic Analysis Using Parsimony) Software version 4.0b4 (Sinauer Associates) and with tree-bisection branch-swapping. The distance model used was Kimura (1980) two-parameter. Relative rate test was performed with the aid of Phyltest program version 2.0 (c).

RESULTS AND DISCUSSION

DNA amplification, sequencing and sequence alignments

A PCR product of the expected size of 884 bp was obtained for *tuf* and of 884 or 871 bp for *atpD* from all bacterial strains tested. After subtracting for biased

primer regions and ambiguous single strand data, sequences of at least 721 bp for *tuf* and 713 bp for *atpD* were submitted to phylogenetic analyses. These sequences were aligned with *tuf* and *atpD* sequences available in databases to verify that the nucleotide sequences indeed encoded a part of tested genes. Gaps were excluded to perform phylogenetic analysis.

Signature sequences

From the sequence alignments obtained from both tested genes, only one insertion was observed. This five amino acids insertion is located between the positions 325 and 326 of *atpD* gene of *E. coli* strain K-12 (Saraste *et al.*, 1981) and can be considered a signature sequence of *Tatumella ptyseos* and *Pantoea* species (Fig. 7). The presence of a conserved indel of defined length and sequence and flanked by conserved regions could suggest a common ancestor, particularly when members of a given taxa share this indel (Gupta, 1998). To our knowledge, high relatedness between the genera *Tatumella* and *Pantoea* is demonstrated for the first time.

Enterobacter agglomerans ATCC 27989 sequence does not possess the five amino acid indel (Fig. 7). This indel could represent a useful marker to help resolve the *Enterobacter agglomerans* and *Pantoea* classification. Indeed, the transfer of *Enterobacter agglomerans* to *Pantoea agglomerans* was proposed in 1989 by Gavini *et al.* (Gavini *et al.*, 1989). However, some strains are provisionally classified as *Pantoea* sp. until their interrelatedness is elucidated (Gavini, *et al.*, 1989). Since the transfer was proposed, the change of nomenclature has not yet been made for all *Enterobacter agglomerans* in the ATCC database. The absence of the five amino acids indel suggests that some strains of *Enterobacter agglomerans* most likely do not belong to the genus *Pantoea*.

Phylogenetic trees based on partial *tuf* sequences, *atpD* sequences, and published 16S rDNA data of members of the *Enterobacteriaceae*.

Representative trees constructed from *tuf* and *atpD* sequences with the neighbor-joining method are shown in Fig. 8. The phylogenetic trees generated from partial *tuf* sequences and *atpD* sequences are very similar. Nevertheless, *atpD* tree shows more monophyletic groups corresponding to species that belong to the same genus. These groups are more consistent with the actual taxonomy. For both genes, some genera are not monophyletic. These results support previous phylogenies based on the genes *gap* and *ompA* (Lawrence, *et al.*, 1991), *rpoB* (Mollet, *et al.*, 1997), and *infB* (Hedegaard, *et al.*, 1999) which all showed that the genera *Escherichia* and *Klebsiella* are polyphyletic. There were few differences in branching between *tuf* and *atpD* genes.

Even though *Pantoea agglomerans* and *Pantoea dispersa* indels were excluded for phylogenetic analysis, these two species grouped together and were distant from *Enterobacter agglomerans* ATCC 27989, adding another evidence that the latter species is heterogenous and that not all members of this species belong to the genus *Pantoea*. In fact, the *E. agglomerans* strain ATCC 27989 exhibits branch lengths similar to others *Enterobacter* species with both genes. Therefore, we suggest that this strain belong to the genus *Enterobacter* until further reclassification of that genus.

tuf and *atpD* trees exhibit very short genetic distances between taxa belonging to the same genetic species including species segregated for clinical considerations. This first concern *E. coli* and *Shigella* species that were confirmed to be the same genetic species by hybridization studies (Brenner *et al.*, 1972; Brenner *et al.*, 1972; Brenner *et al.*, 1982) and phylogenies based on 16S rDNA (Wang *et al.*, 1997) and *rpoB* genes (Mollet, *et al.*, 1997). Hybridization studies (Bercovier, *et al.*, 1980) and phylogeny based on 16S rDNA genes (Ibrahim *et al.*, 1994) demonstrated also that *Yersinia pestis* and *Y. pseudotuberculosis* are the same genetic species. Among

Yersinia pestis and *Y. pseudotuberculosis*, the three *Klebsiella pneumoniae* subspecies, *E. coli-Shigella* species, and *Salmonella choleraesuis* subspecies, *Salmonella* is a less tightly knit species than the other genetic species. The same is true for *E. coli* and *Shigella* species.

Escherichia fergusonii is very close to *E. coli-Shigella* genetic species. This observation is corroborated by 16S rDNA phylogeny (McLaughlin *et al.*, 2000) but not by DNA hybridization values. In fact, *E. fergusonii* is only 49% to 63% related to *E. coli-Shigella* (Farmer III, *et al.*, 1985b). It was previously observed that very recently diverged species may not be recognizable based on 16S rDNA sequences although DNA hybridization established them as different species (Fox *et al.*, 1992). Therefore, *E. fergusonii* could be a new “quasi-species”.

atpD phylogeny revealed *Salmonella* subspecies divisions consistent with the actual taxonomy. This result was already observed by Christensen *et al.* (Christensen & Olsen, 1998). Nevertheless, *tuf* partial sequences discriminate less than *atpD* between *Salmonella* subspecies.

Overall, *tuf* and *atpD* phylogenies exhibit enough divergence between species to ensure efficient discrimination. Therefore, it could be easy to distinguish phenotypically close enterobacteria belonging to different genetic species such as *Klebsiella pneumoniae* and *Enterobacter aerogenes*.

Phylogenetic relationships between *Salmonella*, *E. coli* and *C. freundii* are not well defined. 16S rDNA and 23S rDNA sequence data reveals a closer relationship between *Salmonella* and *E. coli* than between *Salmonella* and *C. freundii* (Christensen *et al.*, 1998), while DNA homology studies (Selander *et al.*, 1996) and *infB* phylogeny (Hedegaard, *et al.*, 1999) showed that *Salmonella* is more closely related to *C. freundii* than to *E. coli*. In that regard, *tuf* and *atpD* phylogenies are coherent with 16S rDNA and 23S rDNA sequence analysis.

Phylogenetic analyses were also performed using amino acids sequences. *tuf* tree based on amino acids is characterized by a better resolution between taxa outgroup and taxa ingroup (enterobacteria) than tree based on nucleic acids whereas *atpD* trees based on amino acids and nucleic acids give almost the same resolution between taxa outgroup and ingroup (data not shown).

Relative rate test (or two cluster test (Takezaki *et al.*, 1995)) evaluates if evolution is constant between two taxa. Before to apply the test, the topology of a tree is determined by tree-building method without the assumption of rate constancy. Therefore, two taxa (or two groups of taxa) are compared with a third taxon that is an outgroup of the first two taxa (Takezaki, *et al.*, 1995). Few pairs of taxa that exhibited a great difference between their branch lengths at particular nodes were chosen to perform the test. This test reveals that *tuf* and *atpD* are not constant in their evolution within the family *Enterobacteriaceae*. For *tuf*, for example, the hypothesis of rate constancy is rejected (Z value higher than 1.96) between *Yersinia* species. The same is true for *Proteus* species. For *atpD*, for example, evolution is not constant between *Proteus* species, between *Proteus* species and *Providencia* species, and between *Yersinia* species and *Escherichia coli*. For 16S rDNA, for example, evolution is not constant between two *E. coli*, between *E. coli* and *Enterobacter aerogenes*, and between *E. coli* and *Proteus vulgaris*. These results suggest that *tuf*, *atpD* and 16S rDNA could not serve as a molecular clock for the entire family *Enterobacteriaceae*.

Since the number and the nature of taxa can influence topology of trees, phylogenetic trees from *tuf* and *atpD* were reconstructed using sequences corresponding to strains for which 16S rDNA genes were published in GenEMBL. These trees were similar to those generated using 16S rDNA (Fig. 9). Nevertheless, 16S rDNA tree gave poorer resolution power than *tuf* and *atpD* gene trees. Indeed, these latter exhibited less multifurcation (polytomy) than the 16S rDNA tree.

Comparison of distances based on *tuf*, *atpD*, and 16S rDNA data.

tuf, *atpD*, and 16S rDNA distances (i.e. the number of differences per nucleotide site) were compared with each other for each pair of strains. We found that the *tuf* and *atpD* distances were respectively 2.268 ± 0.965 and 2.927 ± 0.896 times larger than 16S rDNA distances (Fig. 10a and b). *atpD* distances were 1.445 ± 0.570 times larger than *tuf* distances (Fig. 10c). Figure 10 also shows that the *tuf*, *atpD*, and 16S rDNA distances between members of different species of the same genus (0.053 ± 0.034 , 0.060 ± 0.020 , and 0.024 ± 0.010 , respectively) were in mean smaller than the distances between members of different genera belonging to the same family (0.103 ± 0.053 , 0.129 ± 0.051 , and 0.044 ± 0.013 , respectively). However, the overlap exhibits with standard deviations add to a focus of evidences that some enterobacterial genera are not well defined (Brenner, 1984). In fact, many distances for pairs of species especially belonging to the genera *Escherichia*, *Shigella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Kluyvera* overlap distances for pairs of species belonging to the same genus (Fig. 10). For example, distances for pairs composed by species of *Citrobacter* and species of *Klebsiella* overlap distances for pairs composed by two *Citrobacter* or by two *Klebsiella*.

Observing the distance distributions, 16S rDNA distances reveal a clear separation between the families *Enterobacteriaceae* and *Vibrionaceae* despite the fact that the family *Vibrionaceae* is genetically very close to the *Enterobacteriaceae* (Fig. 10a and b). Nevertheless, *tuf* and *atpD* show higher discriminating power below the family level (Fig. 10a and b).

There were some discrepancies in the relative distances for the same pairs of taxa between the two genes studied. First, distances between *Yersinia* species are at least two times lower for *atpD* than for *tuf* (Fig. 10c). Also, distances at the family level (between *Enterobacteriaceae* and *Vibrionaceae*) show that *Enterobacteriaceae* is a tightlier knit family with *atpD* gene (*Proteus* genus

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excepted) than with *tuf* gene. Both genes well delineate taxa belonging to the same species. There is one exception with *atpD*: *Klebsiella planticola* and *K. ornithinolithica* belong to the same genus but fit with taxa belonging to the same species (Fig. 10a and c). These two species are also very close genotypically with *tuf* gene. This suggest that *Klebsiella planticola* and *K. ornithinolithica* could be two newborn species. *tuf* and *atpD* genes exhibit little distances between *Escherichia fergusonii* and *E. coli-Shigella* species. Unfortunately, comparison with 16S rDNA could not be achieved because the *E. fergusonii* 16S rDNA sequence is not yet accessible in GenEMBL database. Therefore, the majority of phenotypically close enterobacteria could be easily discriminated genotypically using *tuf* and *atpD* gene sequences.

In conclusion, *tuf* and *atpD* genes exhibit phylogenies consistent with 16S rDNA genes phylogeny. For example, they reveal that the family *Enterobacteriaceae* is monophyletic. Moreover, *tuf* and *atpD* distances provide a higher discriminating power than 16S rDNA distances. In fact, *tuf* and *atpD* genes discriminate well between different genospecies and are conserved between strains of the same genetic species in such a way that primers and molecular probes for diagnostic purposes could be designed. Preliminary studies support these observations and diagnostic tests based on *tuf* and *atpD* sequence data to identify enterobacteria are currently under development.

EXAMPLE 44:

Testing new pairs of PCR primers selected from two species-specific genomic DNA fragments which are objects of our assigned US patent 6,001,564

Objective: The goal of these experiments is to demonstrate that it is relatively easy for a person skilled in the art to find other PCR primer pairs from the species-specific

fragments used as targets for detection and identification of a variety of microorganisms. In fact, we wish to prove that the PCR primers previously tested by our group and which are objects of the present patent application are not the only possible good choices for diagnostic purposes. For this example, we used diagnostic targets described in our assigned US patent 6,001,564.

Experimental strategy: We have selected randomly two species-specific genomic DNA fragments for this experiment. The first one is the 705-bp fragment specific to *Staphylococcus epidermidis* (SEQ ID NO: 36 from US patent 6,001,564) while the second one is the 466-bp fragment specific to *Moraxella catarrhalis* (SEQ ID NO: 29 from US patent 6,001,564). Subsequently, we have selected from these two fragments a number of PCR primer pairs other than those previously tested. We have chosen 5 new primer pairs from each of these two sequences which are well dispersed along the DNA fragment (Figures 11 and 12). We have tested these primers for their specificity and compared them with the original primers previously tested. For the specificity tests, we have tested all bacterial species closely related to the target species based on phylogenetic analysis with three conserved genes (rRNA genes, *tuf* and *atpD*). The rationale for selecting a restricted number of bacterial species to evaluate the specificity of the new primer pairs is based on the fact that the lack of specificity of a DNA-based assay is attributable to the detection of closely related species which are more similar at the nucleotide level. Based on the phylogenetic analysis, we have selected (i) species from the closely related genus *Staphylococcus*, *Enterococcus*, *Streptococcus* and *Listeria* to test the specificity of the *S. epidermidis*-specific PCR assays and (ii) species from the closely related genus *Moraxella*, *Kingella* and *Neisseria* to test the specificity of the *M. catarrhalis*-specific PCR assays.

Materials and methods

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Bacterial strains. All bacterial strains used for these experiments were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Genomic DNA isolation. Genomic DNA was purified from the ATCC reference strains by using the G-nome DNA kit (Bio 101 Inc., Vista, CA).

Oligonucleotide design and synthesis. PCR primers were designed with the help of the OligoTM primer analysis software Version 4.0 (National Biosciences Inc., Plymouth, Minn.) and synthesized using a model 391 DNA synthesizer (Applied Biosystems, Foster City, CA).

PCR assays. All PCR assays were performed by using genomic DNA purified from reference strains obtained from the ATCC. One μ l of purified DNA preparation (containing 0.01 to 1 ng of DNA per μ l) was added directly into the PCR reaction mixture. The 20 μ L PCR reactions contained final concentrations of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl₂, 0.4 μ M of each primer, 200 μ M of each of the four dNTPs and 0.5 unit of *Taq* DNA polymerase (Promega, Madison, WI) combined with the TaqStartTM antibody (Clontech Laboratories Inc., Palo Alto, CA). An internal control was integrated into all amplification reactions to verify the efficiency of the amplification reaction as well as to ensure that significant PCR inhibition was absent. Primers amplifying a region of 252 bp from a control plasmid added to each amplification reaction were used to provide the internal control. PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 50 to 65°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc., Watertown, MA). PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 0.25 μ g/mL of ethidium bromide under UV at 254 nm.

Results

Tables 21 and 22 show the results of specificity tests with the 5 new primer pairs selected from SEQ ID NO: 29 (specific to *M. catarrhalis* from US patent 6,001,564) and SEQ ID NO: 36 (specific to *S. epidermidis* from US patent 6,001,564), respectively. In order to evaluate the performance of these new primers pairs, we compared them in parallel with the original primer pairs previously tested.

For *M. catarrhalis*, all of the 5 selected PCR primer pairs were specific for the target species because none of the closely related species could be amplified (Table 21). In fact, the comparison with the original primer pair SEQ ID NO: 118 + SEQ ID NO: 119 (from US patent 6,001,564) revealed that all new pairs showed identical results in terms of specificity and sensitivity thereby suggesting their suitability for diagnostic purposes.

For *S. epidermidis*, 4 of the 5 selected PCR primer pairs were specific for the target species (Table 22). It should be noted that for 3 of these four primer pairs the annealing temperature had to be increased from 55 °C to 60 or 65 °C to attain specificity for *S. epidermidis*. Again the comparison with the original primer pair SEQ ID NO: 145 + SEQ ID NO: 146 (from US patent 6,001,564) revealed that these four primer pairs were as good as the original pair. Increasing the annealing temperature for the PCR amplification is well known by persons skilled in the art to be a very effective way to improve the specificity of a PCR assay (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). In fact, those skilled in the art are well aware of the fact that the annealing temperature is critical for the optimization of PCR assays. Only the primer pair VBsep3 + VBsep4 amplified bacterial species other than *S. epidermidis* including the staphylococcal species *S. capitis*, *S. cohnii*, *S. aureus*, *S. haemolyticus* and *S. hominis* (Table 22). For this non-specific primer pair, increasing the annealing temperature

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from 55 to 65 °C was not sufficient to attain the desired specificity. One possible explanation for the fact that it appears slightly easier to select species-specific primers for *M. catarrhalis* than for *S. epidermidis* is that *M. catarrhalis* is more isolated in phylogenetic trees than *S. epidermidis*. The large number of coagulase negative staphylococcal species such as *S. epidermidis* is largely responsible for this phylogenetic clustering.

Conclusion

These experiment clearly show that it is relatively easy for a person skilled in the art to select, from the species-specific DNA fragments selected as target for identification, PCR primer pairs suitable for diagnostic purposes other than those previously tested. The amplification conditions can be optimize by modifying critical variables such as the annealing temperature to attain the desired specificity and sensitivity. Consequently, we consider that it is legitimate to claim any possible primer sequences selected from the species-specific fragment and that it would be unfair to grant only the claims dealing with the primer pairs previously tested. By extrapolation, these results strongly suggest that it is also relatively easy for a person skilled in the art to select, from the species-specific DNA fragments, DNA probes suitable for diagnostic purposes other than those previously tested.

EXAMPLE 45:

Testing modified versions of PCR primers derived from the sequence of several primers which are objects of US patent 6,001,564.

Objective: The purpose of this project is to verify the efficiency of amplification by modified PCR primers derived from primers previously tested. The types of primer modifications to be tested include (i) variation of the sequence at one or more nucleotide positions and (ii) increasing or reducing the length of the primers. For this example, we used diagnostic targets described in US patent 6,001,564.

Experimental strategy:

a) Testing primers with nucleotide changes

We have designed 13 new primers which are derived from the *S. epidermidis*-specific SEQ ID NO: 146 from US patent 6,001,564 (Table 23). These primers have been modified at one or more nucleotide positions. As shown in Table 23, the nucleotide changes were introduced all along the primer sequence. Furthermore, instead of modifying the primer at any nucleotide position, the nucleotide changes were introduced at the third position of each codon to better reflect potential genetic variations *in vivo*. It should be noted that no nucleotide changes were introduced at the 3' end of the oligonucleotide primers because those skilled in the art are well aware of the fact that mismatches at the 3' end should be avoided (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). All of these modified primers were tested in PCR assays in combination with SEQ ID NO: 145 from US patent 6,001,564 and the efficiency of the amplification was compared with the original primer pair SEQ ID NO: 145 + SEQ ID NO: 146 previously tested in US patent 6,001,564.

b) Testing shorter or longer versions of primers

We have designed shorter and longer versions of the original *S. epidermidis*-specific PCR primer pair SEQ ID NO: 145 + 146 from US patent 6,001,564 (Table 24) as well as shorter versions of the original *P. aeruginosa*-specific primer pair SEQ ID NO: 83 + 84 from US patent 6,001,564 (Table 25). As shown in Tables 24 and 25, both primers of each pair were shortened or lengthen to the same length. Again, those skilled in the art know that the melting temperature of both primers from a pair should be similar to avoid preferential binding at one primer binding site which is

detrimental in PCR (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). All of these shorter or longer primer versions were tested in PCR assays and the efficiency of the amplification was compared with the original primer pair SEQ ID NOs 145 and 146.

Materials and methods

See the Materials and methods section of Example 44.

Results

a) Testing primers with nucleotide changes

The results of the PCR assays with the 13 modified versions of SEQ ID NO: 146 from US patent 6,001,564 are shown in Table 23. The 8 modified primers having a single nucleotide variation showed an efficiency of amplification identical to the original primer pair based on testing with 3 different dilutions of genomic DNA. The four primers having two nucleotide variations and primer VBmut12 having 3 nucleotide changes also showed PCR results identical to those obtained with the original pair. Finally, primer VBmut13 with four nucleotide changes showed a reduction in sensitivity by approximately one log as compared with the original primer pair. However, reducing the annealing temperature from 55 to 50 °C gave an efficiency of amplification very similar to that observed with the original primer pair (Table 23). In fact, reducing the annealing temperature of PCR cycles represents an effective way to reduce the stringency of hybridization for the primers and consequently allows the binding of probes with mismatches (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Subsequently, we have confirmed the

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specificity of the PCR assays with each of these 13 modified versions of SEQ ID NO: 146 from US patent 6,001,564 by performing amplifications from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

b) Testing shorter or longer versions of primers

For these experiments, two primer pairs were selected: i) SEQ ID NO: 145 + 146 from US patent 6,001,564 (specific to *S. epidermidis*) which are AT rich and ii) SEQ ID NO: 83 + 84 (specific to *P. aeruginosa*) which are GC rich. For the AT rich sequence, primers of 15 to 30 nucleotide in length were designed (Table 24) while for the GC rich sequences, primers of 13 to 19 nucleotide in length were designed (Table 25).

Table 24 shows that, for an annealing temperature of 55 °C, the 30-, 25-, 20- and 17-nucleotide versions of SEQ ID NO: 145 and 146 from US patent 6,001,564 all showed identical results as compared with the original primer pair except that the 17-nucleotide version amplified slightly less efficiently the *S. epidermidis* DNA. Reducing the annealing temperature from 55 to 45 °C for the 17-nucleotide version allowed to increase the amplification efficiency to a level very similar to that with the original primer pair (SEQ ID NO: 145 + 146 from US patent 6,001,564). Regarding the 15-nucleotide version, there was amplification of *S. epidermidis* DNA only when the annealing temperature was reduced to 45 °C. Under those PCR conditions the assay remained *S. epidermidis*-specific but the amplification signal with *S. epidermidis* DNA was slightly lower as compared with the original primer pair. Subsequently, we have further confirmed the specificity of the shorter or longer versions by amplifying DNA from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

Table 25 shows that, for an annealing temperature of 55 °C, all shorter versions of SEQ ID NO: 83 and 84 from US patent 6,001,564 showed identical PCR results as

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compared with the original primer pair. As expected, these results show that it is simpler to reduce the length of GC rich as compared with AT rich. This is attributable to the fact that GC binding is more stable than AT binding.

Conclusion

a) Testing primers with nucleotide changes

The above experiments clearly show that PCR primers may be modified at one or more nucleotide positions without affecting the specificity and the sensitivity of the PCR assay. These results strongly suggest that a given oligonucleotide can detect variant genomic sequences from the target species. In fact, the nucleotide changes in the selected primers were purposely introduced at the third position of each codon to mimic nucleotide variation in genomic DNA. Thus we conclude that it is justified to claim "a variant thereof" for i) the SEQ IDs of the fragments and oligonucleotides which are object of the present patent application and ii) genomic variants of the target species.

b) Testing shorter or longer versions of primers

The above experiments clearly show that PCR primers may be shorter or longer without affecting the specificity and the sensitivity of the PCR assay. We have showed that oligonucleotides ranging in sizes from 13 to 30 nucleotides may be as specific and sensitive as the original primer pair from which they were derived. Consequently, these results suggest that it is not exaggerated to claim sequences having at least 12 nucleotide in length.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

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Table 1. Distribution (%) of nosocomial pathogens for various human infections in USA (1990-1992)¹.

Pathogen	UTI ²	SSI ³	BSI ⁴	Pneumonia	CSF ⁵
<i>Escherichia coli</i>	27	9	5	4	2
<i>Staphylococcus aureus</i>	2	21	17	21	2
<i>Staphylococcus epidermidis</i>	2	6	20	0	1
<i>Enterococcus faecalis</i>	16	12	9	2	0
<i>Enterococcus faecium</i>	1	1	0	0	0
<i>Pseudomonas aeruginosa</i>	12	9	3	18	0
<i>Klebsiella pneumoniae</i>	7	3	4	9	0
<i>Proteus mirabilis</i>	5	3	1	2	0
<i>Streptococcus pneumoniae</i>	0	0	3	1	18
Group B <i>Streptococci</i>	1	1	2	1	6
Other streptococci	3	5	2	1	3
<i>Haemophilus influenzae</i>	0	0	0	6	45
<i>Neisseria meningitidis</i>	0	0	0	0	14
<i>Listeria monocytogenes</i>	0	0	0	0	3
Other enterococci	1	1	0	0	0
Other staphylococci	2	8	13	2	0
<i>Candida albicans</i>	9	3	5	5	0
Other <i>Candida</i>	2	1	3	1	0
<i>Enterobacter</i> sp.	5	7	4	12	2
<i>Acinetobacter</i> sp.	1	1	2	4	2
<i>Citrobacter</i> sp.	2	1	1	1	0
<i>Serratia marcescens</i>	1	1	1	3	1
Other <i>Klebsiella</i>	1	1	1	2	1
Others	0	6	4	5	0

¹ Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, 6:428-442).

² Urinary tract infection.

³ Surgical site infection.

⁴ Bloodstream infection.

⁵ Cerebrospinal fluid.

Table 2. Distribution (%) of bloodstream infection pathogens in Quebec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

5	Organism	Quebec ¹	Canada ²	UK ³	USA ⁴	
				Community-acquired	Hospital-acquired	Hospital-acquired
	<i>E. coli</i>	15.6	53.8	24.8	20.3	5.0
10	<i>S. epidermidis</i> and other CoNS ⁵	25.8	-	0.5	7.2	31.0
	<i>S. aureus</i>	9.6	-	9.7	19.4	16.0
	<i>S. pneumoniae</i>	6.3	-	22.5	2.2	-
	<i>E. faecalis</i>	3.0	-	1.0	4.2	-
15	<i>E. faecium</i>	2.6	-	0.2	0.5	-
	<i>Enterococcus</i> sp.	-	-	-	9.0	-
	<i>H. influenzae</i>	1.5	-	3.4	0.4	-
	<i>P. aeruginosa</i>	1.5	8.2	1.0	8.2	3.0
	<i>K. pneumoniae</i>	3.0	11.2	3.0	9.2	4.0
20	<i>P. mirabilis</i>	-	3.9	2.8	5.3	1.0
	<i>S. pyogenes</i>	-	-	1.9	0.9	-
	<i>Enterobacter</i> sp.	4.1	5.5	0.5	2.3	4.0
	<i>Candida</i> sp.	8.5	-	-	1.0	8.0
	Others	18.5	17.4	28.7	18.9	19.0

25 ¹ Data obtained for 270 isolates collected at the Centre Hospitalier de l'Université Laval (CHUL) during a 5 month period (May to October 1995).

² Data from 10 hospitals throughout Canada representing 941 gram-negative isolates. (Chamberland *et al.*, 1992, *Clin. Infect. Dis.*, **15**:615-628).

30 ³ Data from a 20-year study (1969-1988) for nearly 4000 isolates. (Eykyn *et al.*, 1990, *J. Antimicrob. Chemother.*, Suppl. C, **25**:41-58).

⁴ Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, **6**:428-442).

⁵ Coagulase-negative staphylococci.

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Table 3. Distribution of positive and negative clinical specimens tested at the microbiology laboratory of the CHUL (February 1994 – January 1995).

5	Clinical specimens and/or sites	No. of samples tested (%)	% of positive specimens	% of negative specimens
	Urine	17,981 (54.5)	19.4	80.6
	Blood culture/marrow	10,010 (30.4)	6.9	93.1
	Sputum	1,266 (3.8)	68.4	31.6
10	Superficial pus	1,136 (3.5)	72.3	27.7
	Cerebrospinal fluid	553 (1.7)	1.0	99.0
	Synovial fluid	523 (1.6)	2.7	97.3
	Respiratory tract	502 (1.5)	56.6	43.4
	Deep pus	473 (1.4)	56.8	43.2
15	Ears	289 (0.9)	47.1	52.9
	Pleural and pericardial fluid	132 (0.4)	1.0	99.0
	Peritoneal fluid	101(0.3)	28.6	71.4
	Total:	32,966 (100.0)	20.0	80.0

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention.

5	Bacterial species	
	<i>Abiotrophia adiacens</i>	<i>Brevibacterium flavum</i>
	<i>Abiotrophia defectiva</i>	<i>Brevundimonas diminuta</i>
	<i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i>	65 <i>Buchnera aphidicola</i>
10	<i>Acetobacterium woodii</i>	<i>Budvicia aquatica</i>
	<i>Acetobacter acetii</i>	<i>Burkholderia cepacia</i>
	<i>Acetobacter altoacetigenes</i>	<i>Burkholderia mallei</i>
	<i>Acetobacter polyoxogenes</i>	<i>Burkholderia pseudomallei</i>
	<i>Acholeplasma laidlawii</i>	70 <i>Buttiauxella agrestis</i>
15	<i>Acidothermus cellulolyticus</i>	<i>Butyrivibrio fibrisolvens</i>
	<i>Acidiphilum facilis</i>	<i>Campylobacter coli</i>
	<i>Acinetobacter baumannii</i>	<i>Campylobacter curvus</i>
	<i>Acinetobacter calcoaceticus</i>	<i>Campylobacter fetus</i> subsp. <i>fetus</i>
	<i>Acinetobacter lwoffii</i>	75 <i>Campylobacter fetus</i> subsp. <i>venerealis</i>
20	<i>Actinomyces meyeri</i>	<i>Campylobacter gracilis</i>
	<i>Aerococcus viridans</i>	<i>Campylobacter jejuni</i>
	<i>Aeromonas hydrophila</i>	<i>Campylobacter jejuni</i> subsp. <i>doylei</i>
	<i>Aeromonas salmonicida</i>	<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>
	<i>Agrobacterium radiobacter</i>	80 <i>Campylobacter lari</i>
25	<i>Agrobacterium tumefaciens</i>	<i>Campylobacter rectus</i>
	<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	<i>Campylobacter sputorum</i> subsp. <i>sputorum</i>
	<i>Allochromatium vinosum</i>	<i>Campylobacter upsaliensis</i>
	<i>Anabaena variabilis</i>	<i>Cedecea davisae</i>
	<i>Anacystis nidulans</i>	85 <i>Cedecea lapagei</i>
30	<i>Anaerorhabdus furcosus</i>	<i>Cedecea neteri</i>
	<i>Aquifex aeolicus</i>	<i>Chlamydia pneumoniae</i>
	<i>Aquifex pyrophilus</i>	<i>Chlamydia psittaci</i>
	<i>Arcanobacterium haemolyticum</i>	<i>Chlamydia trachomatis</i>
	<i>Archaeoglobus fulgidus</i>	90 <i>Chlorobium vibrioforme</i>
35	<i>Azotobacter vinelandii</i>	<i>Chloroflexus aurantiacus</i>
	<i>Bacillus anthracis</i>	<i>Chryseobacterium meningosepticum</i>
	<i>Bacillus cereus</i>	<i>Citrobacter amalonaticus</i>
	<i>Bacillus firmus</i>	<i>Citrobacter braakii</i>
	<i>Bacillus halodurans</i>	95 <i>Citrobacter farmeri</i>
40	<i>Bacillus megaterium</i>	<i>Citrobacter freundii</i>
	<i>Bacillus mycoides</i>	<i>Citrobacter koseri</i>
	<i>Bacillus pseudomycoides</i>	<i>Citrobacter sedlakii</i>
	<i>Bacillus stearothermophilus</i>	<i>Citrobacter werkmanii</i>
	<i>Bacillus subtilis</i>	100 <i>Citrobacter youngae</i>
45	<i>Bacillus thuringiensis</i>	<i>Clostridium acetobutylicum</i>
	<i>Bacillus weihenstephanensis</i>	<i>Clostridium beijerinckii</i>
	<i>Bacteroides distasonis</i>	<i>Clostridium bifermentans</i>
	<i>Bacteroides fragilis</i>	<i>Clostridium botulinum</i>
	<i>Bacteroides forsythus</i>	105 <i>Clostridium difficile</i>
50	<i>Bacteroides ovatus</i>	<i>Clostridium innocuum</i>
	<i>Bacteroides vulgatus</i>	<i>Clostridium histolyticum</i>
	<i>Bartonella henselae</i>	<i>Clostridium novyi</i>
	<i>Bifidobacterium adolescentis</i>	<i>Clostridium septicum</i>
	<i>Bifidobacterium breve</i>	110 <i>Clostridium perfringens</i>
55	<i>Bifidobacterium dentium</i>	<i>Clostridium ramosum</i>
	<i>Bifidobacterium longum</i>	<i>Clostridium sordellii</i>
	<i>Blastochloris viridis</i>	<i>Clostridium tertium</i>
	<i>Borrelia burgdorferi</i>	<i>Clostridium tetani</i>
	<i>Bordetella pertussis</i>	115 <i>Comamonas acidovorans</i>
60	<i>Bordetella bronchiseptica</i>	<i>Corynebacterium accolens</i>
	<i>Brucella abortus</i>	<i>Corynebacterium bovis</i>
	<i>Brevibacterium linens</i>	<i>Corynebacterium cervicis</i>

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

Bacterial species (continued)	
5	<i>Corynebacterium diphtheriae</i>
	<i>Corynebacterium flavescens</i>
	<i>Corynebacterium genitalium</i>
	<i>Corynebacterium glutamicum</i>
10	<i>Corynebacterium jeikeium</i>
	<i>Corynebacterium kutscheri</i>
	<i>Corynebacterium minutissimum</i>
	<i>Corynebacterium mycetoides</i>
	<i>Corynebacterium pseudodiphtheriticum</i>
15	<i>Corynebacterium pseudogenitalium</i>
	<i>Corynebacterium pseudotuberculosis</i>
	<i>Corynebacterium renale</i>
	<i>Corynebacterium striatum</i>
	<i>Corynebacterium ulcerans</i>
20	<i>Corynebacterium urealyticum</i>
	<i>Corynebacterium xerosis</i>
	<i>Coxiella burnetii</i>
	<i>Cytophaga lytica</i>
	<i>Deinococcus radiodurans</i>
25	<i>Deinonema</i> sp.
	<i>Edwardsiella hoshinae</i>
	<i>Edwardsiella tarda</i>
	<i>Ehrlichia canis</i>
	<i>Ehrlichia risticii</i>
30	<i>Eikenella corrodens</i>
	<i>Enterobacter aerogenes</i>
	<i>Enterobacter agglomerans</i>
	<i>Enterobacter amnigenus</i>
	<i>Enterobacter asburiae</i>
35	<i>Enterobacter cancerogenus</i>
	<i>Enterobacter cloacae</i>
	<i>Enterobacter gergoviae</i>
	<i>Enterobacter hormaechei</i>
	<i>Enterobacter sakazakii</i>
40	<i>Enterococcus avium</i>
	<i>Enterococcus casseliflavus</i>
	<i>Enterococcus cecorum</i>
	<i>Enterococcus columbae</i>
	<i>Enterococcus dispar</i>
45	<i>Enterococcus durans</i>
	<i>Enterococcus faecalis</i>
	<i>Enterococcus faecium</i>
	<i>Enterococcus flavescens</i>
	<i>Enterococcus gallinarum</i>
50	<i>Enterococcus hirae</i>
	<i>Enterococcus malodoratus</i>
	<i>Enterococcus mundtii</i>
	<i>Enterococcus pseudoavium</i>
	<i>Enterococcus raffinosus</i>
55	<i>Enterococcus saccharolyticus</i>
	<i>Enterococcus solitarius</i>
	<i>Enterococcus sulfureus</i>
	<i>Erwinia amylovora</i>
	<i>Erwinia carotovora</i>
60	<i>Escherichia coli</i>
	<i>Escherichia fergusonii</i>
	<i>Escherichia hermannii</i>
	<i>Escherichia vulneris</i>
	<i>Eubacterium lentum</i>
65	<i>Eubacterium nodatum</i>
	<i>Ewingella americana</i>
	<i>Francisella tularensis</i>
	<i>Frankia alni</i>
	<i>Fervidobacterium islandicum</i>
70	<i>Fibrobacter succinogenes</i>
	<i>Flavobacterium ferrugineum</i>
	<i>Flexistipes sinusarabici</i>
	<i>Fusobacterium gonidiaformans</i>
	<i>Fusobacterium necrophorum</i> subsp. <i>necrophorum</i>
75	<i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>
	<i>Gardnerella vaginalis</i>
	<i>Gemella haemolysans</i>
	<i>Gemella morbillorum</i>
	<i>Globicatella sanguis</i>
80	<i>Gloeobacter violaceus</i>
	<i>Gloeotheca</i> sp.
	<i>Gluconobacter oxydans</i>
	<i>Haemophilus actinomycetemcomitans</i>
	<i>Haemophilus aphrophilus</i>
85	<i>Haemophilus ducreyi</i>
	<i>Haemophilus haemolyticus</i>
	<i>Haemophilus influenzae</i>
	<i>Haemophilus parahaemolyticus</i>
	<i>Haemophilus parainfluenzae</i>
90	<i>Haemophilus paraprofundus</i>
	<i>Haemophilus segnis</i>
	<i>Hafnia alvei</i>
	<i>Halobacterium marismortui</i>
	<i>Halobacterium salinarum</i>
95	<i>Haloferax volcanii</i>
	<i>Helicobacter pylori</i>
	<i>Herpetosiphon aurantiacus</i>
	<i>Kingella kingae</i>
	<i>Klebsiella ornithinolytica</i>
100	<i>Klebsiella oxytoca</i>
	<i>Klebsiella planticola</i>
	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>
	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>
	<i>Klebsiella pneumoniae</i> subsp.
105	<i>rhinoscleromatis</i>
	<i>Klebsiella terrigena</i>
	<i>Kluyvera ascorbata</i>
	<i>Kluyvera cryocrescens</i>
	<i>Kluyvera georgiana</i>
110	<i>Kocuria kristinae</i>
	<i>Lactobacillus acidophilus</i>
	<i>Lactobacillus garvieae</i>
	<i>Lactobacillus paracasei</i>
	<i>Lactobacillus casei</i> subsp. <i>casei</i>
115	<i>Lactococcus garvieae</i>
	<i>Lactococcus lactis</i>
	<i>Lactococcus lactis</i> subsp. <i>lactis</i>
	<i>Leclercia adecarboxylata</i>
	<i>Legionella micdadei</i>

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

5	Bacterial species (continued)	
	<i>Legionella pneumophila</i> subsp. <i>pneumophila</i>	<i>Neisseria gonorrhoeae</i>
	<i>Leminorella grimontii</i>	<i>Neisseria lactamica</i>
	<i>Leminorella richardii</i>	65 <i>Neisseria meningitidis</i>
10	<i>Leptospira biflexa</i>	<i>Neisseria mucosa</i>
	<i>Leptospira interrogans</i>	<i>Neisseria perflava</i>
	<i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>	<i>Neisseria pharyngis</i> var. <i>flava</i>
	<i>Listeria innocua</i>	<i>Neisseria polysaccharea</i>
	15 <i>Listeria ivanovii</i>	70 <i>Neisseria sicca</i>
	<i>Listeria monocytogenes</i>	<i>Neisseria subflava</i>
	<i>Listeria seeligeri</i>	<i>Neisseria weaveri</i>
	<i>Macrococcus caseolyticus</i>	<i>Obesumbacterium proteus</i>
	<i>Magnetospirillum magnetotacticum</i>	<i>Ochrobactrum anthropi</i>
20	<i>Megamonas hypermegale</i>	75 <i>Pantoea agglomerans</i>
	<i>Methanobacterium thermoautotrophicum</i>	<i>Pantoea dispersa</i>
	<i>Methanococcus jannaschii</i>	<i>Paracoccus denitrificans</i>
	<i>Methanococcus vannielii</i>	<i>Pasteurella multocida</i>
	<i>Methanosarcina barkeri</i>	<i>Pectinatus frisingensis</i>
	25 <i>Methanosarcina jannaschii</i>	80 <i>Peptococcus niger</i>
	<i>Methylobacillus flagellatum</i>	<i>Peptostreptococcus anaerobius</i>
	<i>Methylomonas clara</i>	<i>Peptostreptococcus asaccharolyticus</i>
	<i>Micrococcus luteus</i>	<i>Peptostreptococcus prevotii</i>
	<i>Micrococcus lylae</i>	<i>Phormidium ectocarpi</i>
30	<i>Mitsuokella multacidus</i>	85 <i>Pirellula marina</i>
	<i>Mobiluncus curtisii</i> subsp. <i>holmesii</i>	<i>Planobispora rosea</i>
	<i>Moellerella thermoacetica</i>	<i>Plesiomonas shigelloides</i>
	<i>Moellerella wisconsensis</i>	<i>Plectonema boryanum</i>
	<i>Moorella thermoacetica</i>	<i>Porphyromonas asaccharolytica</i>
35	<i>Moraxella catarrhalis</i>	90 <i>Porphyromonas gingivalis</i>
	<i>Moraxella osloensis</i>	<i>Pragia fontium</i>
	<i>Morganella morganii</i> subsp. <i>morganii</i>	<i>Prevotella buccalis</i>
	<i>Mycobacterium avium</i>	<i>Prevotella melaninogenica</i>
	<i>Mycobacterium bovis</i>	<i>Prevotella oralis</i>
40	<i>Mycobacterium gordonae</i>	95 <i>Prevotella ruminicola</i>
	<i>Mycobacterium kansasii</i>	<i>Prochlorothrix hollandica</i>
	<i>Mycobacterium leprae</i>	<i>Propionibacterium acnes</i>
	<i>Mycobacterium terrae</i>	<i>Propionigenium modestum</i>
	<i>Mycobacterium tuberculosis</i>	<i>Proteus mirabilis</i>
45	<i>Mycoplasma capricolum</i>	100 <i>Proteus penneri</i>
	<i>Mycoplasma gallisepticum</i>	<i>Proteus vulgaris</i>
	<i>Mycoplasma genitalium</i>	<i>Providencia alcalifaciens</i>
	<i>Mycoplasma hominis</i>	<i>Providencia rettgeri</i>
	<i>Mycoplasma pirum</i>	<i>Providencia rustigianii</i>
50	<i>Mycoplasma mycoides</i>	105 <i>Providencia stuartii</i>
	<i>Mycoplasma pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
	<i>Mycoplasma pulmonis</i>	<i>Pseudomonas fluorescens</i>
	<i>Mycoplasma salivarium</i>	<i>Pseudomonas putida</i>
	<i>Myxococcus xanthus</i>	<i>Pseudomonas stutzeri</i>
55	<i>Neisseria animalis</i>	110 <i>Psychrobacter phenylpyruvicum</i>
	<i>Neisseria canis</i>	<i>Pyrococcus abyssi</i>
	<i>Neisseria cinerea</i>	<i>Rahnella aquatilis</i>
	<i>Neisseria cuniculi</i>	<i>Rickettsia prowazekii</i>
	<i>Neisseria elongata</i> subsp. <i>elongata</i>	<i>Rhizobium leguminosarum</i>
60	<i>Neisseria elongata</i> subsp. <i>intermedia</i>	115 <i>Rhizobium phaseoli</i>
	<i>Neisseria flava</i>	<i>Rhodobacter capsulatus</i>
	<i>Neisseria flavescens</i>	<i>Rhodobacter sphaeroides</i>

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

5	Bacterial species (continued)	
10	<i>Rhodopseudomonas palustris</i> <i>Rhodospirillum rubrum</i> <i>Ruminococcus albus</i> <i>Ruminococcus bromii</i> <i>Salmonella bongori</i> <i>Salmonella choleraesuis</i> subsp. <i>arizonae</i> <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> <i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i> <i>Salmonella choleraesuis</i> subsp. <i>houtenae</i> <i>Salmonella choleraesuis</i> subsp. <i>indica</i> <i>Salmonella choleraesuis</i> subsp. <i>salamae</i> <i>Serpulina hyodysenteriae</i> <i>Serratia ficaria</i> <i>Serratia fonticola</i> <i>Serratia grimesii</i> <i>Serratia liquefaciens</i> <i>Serratia marcescens</i> <i>Serratia odorifera</i> <i>Serratia plymuthica</i> <i>Serratia rubidaea</i> <i>Shewanella putrefaciens</i> <i>Shigella boydii</i> <i>Shigella dysenteriae</i> <i>Shigella flexneri</i> <i>Shigella sonnei</i> <i>Sinorhizobium meliloti</i> <i>Spirochaeta aurantia</i> <i>Staphylococcus aureus</i> <i>Staphylococcus aureus</i> subsp. <i>aureus</i> <i>Staphylococcus auricularis</i> <i>Staphylococcus capitis</i> subsp. <i>capitis</i> <i>Staphylococcus cohnii</i> subsp. <i>cohnii</i> <i>Staphylococcus epidermidis</i> <i>Staphylococcus haemolyticus</i> <i>Staphylococcus hominis</i> <i>Staphylococcus hominis</i> subsp. <i>hominis</i> <i>Staphylococcus lugdunensis</i> <i>Staphylococcus saprophyticus</i> <i>Staphylococcus sciuri</i> subsp. <i>sciuri</i> <i>Staphylococcus simulans</i> <i>Staphylococcus warneri</i> <i>Stigmatella aurantiaca</i> <i>Stenotrophomonas maltophilia</i> <i>Streptococcus acidominimus</i> <i>Streptococcus agalactiae</i> <i>Streptococcus anginosus</i> <i>Streptococcus bovis</i> <i>Streptococcus cricetus</i> <i>Streptococcus cristatus</i> <i>Streptococcus downei</i> <i>Streptococcus dysgalactiae</i> <i>Streptococcus equi</i> subsp. <i>equi</i> <i>Streptococcus ferus</i>	65 70 75 80 85 90 95 100 105 110 115
	<i>Streptococcus gordonii</i> <i>Streptococcus macacae</i> <i>Streptococcus mitis</i> <i>Streptococcus mutans</i> <i>Streptococcus oralis</i> <i>Streptococcus parasanguinis</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus pyogenes</i> <i>Streptococcus rattii</i> <i>Streptococcus salivarius</i> <i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> <i>Streptococcus sanguinis</i> <i>Streptococcus sobrinus</i> <i>Streptococcus suis</i> <i>Streptococcus uberis</i> <i>Streptococcus vestibularis</i> <i>Streptomyces anbofaciens</i> <i>Streptomyces aureofaciens</i> <i>Streptomyces cinnamomeus</i> <i>Streptomyces coelicolor</i> <i>Streptomyces collinus</i> <i>Streptomyces lividans</i> <i>Streptomyces netropsis</i> <i>Streptomyces ramocissimus</i> <i>Streptomyces rimosus</i> <i>Streptomyces venezuelae</i> <i>Succinivibrio dextrinosolvens</i> <i>Synechococcus</i> sp. <i>Synechocystis</i> sp. <i>Tatumella ptyseos</i> <i>Taxobacter occealus</i> <i>Tetragenococcus halophilus</i> <i>Thermoplasma acidophilum</i> <i>Thermotoga maritima</i> <i>Thermus aquaticus</i> <i>Thermus thermophilus</i> <i>Thiobacillus ferrooxidans</i> <i>Thiomonas cuprina</i> <i>Trabulsiella guamensis</i> <i>Treponema pallidum</i> <i>Ureaplasma urealyticum</i> <i>Veillonella parvula</i> <i>Vibrio alginolyticus</i> <i>Vibrio anguillarum</i> <i>Vibrio cholerae</i> <i>Vibrio mimicus</i> <i>Wolinella succinogenes</i> <i>Xanthomonas citri</i> <i>Xanthomonas oryzae</i> <i>Xenorhabdus bovienii</i> <i>Xenorhabdus nematophilus</i> <i>Yersinia bercovieri</i> <i>Yersinia enterocolitica</i> <i>Yersinia frederiksenii</i> <i>Yersinia intermedia</i> <i>Yersinia pestis</i>	

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

5	Bacterial species (continued)
	<i>Yersinia pseudotuberculosis</i>
	<i>Yersinia rohdei</i>
	<i>Yokenella regensburgei</i>
10	<i>Zoogloea ramigera</i>

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

5	Fungal species
	<i>Absidia corymbifera</i>
	<i>Absidia glauca</i>
	<i>Alternaria alternata</i>
10	<i>Arxula adenivorans</i>
	<i>Aspergillus flavus</i>
	<i>Aspergillus fumigatus</i>
	<i>Aspergillus nidulans</i>
	<i>Aspergillus niger</i>
15	<i>Aspergillus oryzae</i>
	<i>Aspergillus terreus</i>
	<i>Aspergillus versicolor</i>
	<i>Aureobasidium pullulans</i>
	<i>Basidiobolus ranarum</i>
20	<i>Bipolaris hawaiiensis</i>
	<i>Bilophila wadsworthia</i>
	<i>Blastoschizomyces capitatus</i>
	<i>Blastomyces dermatitidis</i>
	<i>Candida albicans</i>
25	<i>Candida catenulata</i>
	<i>Candida dubliniensis</i>
	<i>Candida famata</i>
	<i>Candida glabrata</i>
	<i>Candida guilliermondii</i>
30	<i>Candida haemulonii</i>
	<i>Candida inconspicua</i>
	<i>Candida kefyr</i>
	<i>Candida krusei</i>
	<i>Candida lambica</i>
35	<i>Candida lusitanae</i>
	<i>Candida norvegica</i>
	<i>Candida norvegensis</i>
	<i>Candida parapsilosis</i>
	<i>Candida rugosa</i>
40	<i>Candida sphaerica</i>
	<i>Candida tropicalis</i>
	<i>Candida utilis</i>
	<i>Candida viswanathii</i>
	<i>Candida zeylanoides</i>
45	<i>Cladophialophora carrionii</i>
	<i>Coccidioides immitis</i>
	<i>Coprinus cinereus</i>
	<i>Cryptococcus albidus</i>
	<i>Cryptococcus humicolus</i>
50	<i>Cryptococcus laurentii</i>
	<i>Cryptococcus neoformans</i>
	<i>Cunninghamella bertholletiae</i>
	<i>Curvularia lunata</i>
	<i>Emericella nidulans</i>
55	<i>Emmonsia parva</i>
	<i>Eremothecium gossypii</i>
	<i>Exophiala dermatitidis</i>
	<i>Exophiala jeanselmei</i>
	<i>Exophiala moniliae</i>
60	<i>Exserohilum rostratum</i>
	<i>Eremothecium gossypii</i>
	<i>Fonsecaea pedrosoi</i>
	<i>Fusarium moniliforme</i>
	<i>Fusarium oxysporum</i>
65	<i>Fusarium solani</i>
	<i>Geotrichum</i> sp.
	<i>Histoplasma capsulatum</i>
	<i>Hortaea werneckii</i>
	<i>Issatchenkia orientalis</i> Kudrjanzev
70	<i>Kluyveromyces lactis</i>
	<i>Malassezia furfur</i>
	<i>Malassezia pachydermatis</i>
	<i>Malbranchea filamentosa</i>
	<i>Metschnikowia pulcherrima</i>
75	<i>Microsporium audouinii</i>
	<i>Microsporium canis</i>
	<i>Mucor circinelloides</i>
	<i>Neurospora crassa</i>
	<i>Paecilomyces lilacinus</i>
80	<i>Paracoccidioides brasiliensis</i>
	<i>Penicillium marneffei</i>
	<i>Phialophora verrucosa</i>
	<i>Pichia anomala</i>
	<i>Piedraia hortai</i>
85	<i>Podospora anserina</i>
	<i>Podospora curvicolla</i>
	<i>Puccinia graminis</i>
	<i>Pseudallescheria boydii</i>
	<i>Reclinomonas americana</i>
90	<i>Rhizomucor racemosus</i>
	<i>Rhizopus oryzae</i>
	<i>Rhodotorula minuta</i>
	<i>Rhodotorula mucilaginosa</i>
	<i>Saccharomyces cerevisiae</i>
95	<i>Saksenaea vasiformis</i>
	<i>Schizosaccharomyces pombe</i>
	<i>Scopulariopsis koningii</i>
	<i>Sordaria macrospora</i>
	<i>Sporobolomyces salmonicolor</i>
100	<i>Sporothrix schenckii</i>
	<i>Stephanoascus ciferrii</i>
	<i>Syncephalastrum racemosum</i>
	<i>Trichoderma reesei</i>
	<i>Trichophyton mentagrophytes</i>
105	<i>Trichophyton rubrum</i>
	<i>Trichophyton tonsurans</i>
	<i>Trichosporon cutaneum</i>
	<i>Ustilago maydis</i>
	<i>Wangiella dermatitidis</i>
110	<i>Yarrowia lipolytica</i>

Table 4. Example of microbial species for which *tuf* and/or *atpD* and/or *recA* nucleic acids and/or sequences are used in the present invention (continued).

Parasitical species	
5	<i>Babesia bigemina</i> <i>Babesia bovis</i> <i>Babesia microti</i> <i>Blastocystis hominis</i>
10	<i>Crithidia fasciculata</i> <i>Cryptosporidium parvum</i> <i>Entamoeba histolytica</i> <i>Giardia lamblia</i> <i>Kentrophoros sp.</i>
15	<i>Leishmania aethiopica</i> <i>Leishmania amazonensis</i> <i>Leishmania braziliensis</i> <i>Leishmania donovani</i> <i>Leishmania infantum</i>
20	<i>Leishmania enriettii</i> <i>Leishmania gerbilli</i> <i>Leishmania guyanensis</i> <i>Leishmania hertigi</i> <i>Leishmania major</i>
25	<i>Leishmania mexicana</i> <i>Leishmania panamensis</i> <i>Leishmania tarentolae</i> <i>Leishmania tropica</i> <i>Neospora caninum</i>
30	<i>Onchocerca volvulus</i> <i>Plasmodium berghei</i> <i>Plasmodium falciparum</i> <i>Plasmodium knowlesi</i> <i>Porphyra purpurea</i>
35	<i>Toxoplasma gondii</i> <i>Treponema pallidum</i> <i>Trichomonas tenax</i> <i>Trichomonas vaginalis</i> <i>Trypanosoma brucei</i>
40	<i>Trypanosoma brucei</i> subsp. <i>brucei</i> <i>Trypanosoma congolense</i> <i>Trypanosoma cruzi</i>

Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes.

	Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5	<i>aac(3)-Ib</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> <i>Pseudomonads</i>	L06157	
	<i>aac(3)-IIb</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	M97172	
	<i>aac(3)-IVa</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i>	X01385	
10	<i>aac(3)-VIa</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	M88012	
	<i>aac(2')-Ia</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	X04555	
	<i>aac(6')-aph(2'')</i> ²	Aminoglycosides	<i>Enterococcus</i> sp., <i>Staphylococcus</i> sp.		83-86 ³
15	<i>aac(6')-Ia</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	M18967	
	<i>aac(6')-Ic</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	M94066	
20	<i>aac(6')-IIa</i> ²	Aminoglycosides	<i>Pseudomonads</i>		112 ⁴
	<i>aadB</i> [<i>ant(2'')-Ia</i> ²]	Aminoglycosides	<i>Enterobacteriaceae</i>		53-54 ³
	<i>aacC1</i> [<i>aac(3)-Ia</i> ²]	Aminoglycosides	<i>Pseudomonads</i>		55-56 ³
	<i>aacC2</i> [<i>aac(3)-IIa</i> ²]	Aminoglycosides	<i>Pseudomonads</i>		57-58 ³
	<i>aacC3</i> [<i>aac(3)-III</i> ²]	Aminoglycosides	<i>Pseudomonads</i>		59-60 ³
25	<i>aacA4</i> [<i>aac(6')-Ib</i> ²]	Aminoglycosides	<i>Pseudomonads</i>		65-66 ³
	<i>ant(3'')-Ia</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Enterococcus</i> sp., <i>Staphylococcus</i> sp.	X02340 M10241	
	<i>ant(4')-Ia</i> ²	Aminoglycosides	<i>Staphylococcus</i> sp.	V01282	
30	<i>aph(3')-Ia</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	J01839	
	<i>aph(3')-IIa</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	V00618	
	<i>aph(3')-IIIa</i> ²	Aminoglycosides	<i>Enterococcus</i> sp., <i>Staphylococcus</i> sp.	V01547	
35	<i>aph(3')-VIa</i> ²	Aminoglycosides	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	X07753	
	<i>rpsL</i> ²	Streptomycin	<i>M. tuberculosis</i> , <i>M. avium</i> complex	X80120 U14749 X70995 L08011	
40					
	<i>bla_{OXA}</i> ^{5,6}	β-lactams	<i>Enterobacteriaceae</i> , <i>Pseudomonads</i>	Y10693 AJ238349 AJ009819 X06046 X03037 X07260 U13880 X75562 AF034958 J03427 Z22590 U59183 L38523 U63835 AF043100 AF060206 U85514 AF043381 AF024602 AF064820	110 ⁴
45					
50					
55					
60					
	<i>bla_{ROB}</i> ⁵	β-lactams	<i>Haemophilus</i> sp. <i>Pasteurella</i> sp.		45-48 ³
65					

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Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5 <i>bla_{SHV}</i> ^{5,6}	β-lactams	<i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i>	AF124984 AF148850 M59181 X98099 M33655 AF148851 X53433 L47119 AF074954 X53817 AF096930 X55640 Y11069 U20270 U92041 S82452 X98101 X98105 AF164577 AJ011428 AF116855 AB023477 AF293345 AF227204 AF208796 AF132290 AF012911 U48775 AF093512 AF052748 X64523 Y13612 X57972 AF157413 U31280 U36911 U48775 V00613 X97254 AJ012256 X04515 AF126482 U09188 M88143 Y14574 AF188200 AJ251946 Y17581 Y17582 Y17583 M88143 U37195 Y17584 X64523 U95363 Y10279 Y10280 Y10281 AF027199 AF104441 AF104442 AF062386 X57972 AF047171 AF188199 AF157553 AF190694 AF190695 AF190693 AF190692	41-44 ³
10				
15				
20				
25				
30				
35	<i>bla_{TEM}</i> ^{5,6}	β-lactams	<i>Enterobacteriaceae</i> , <i>Neisseria</i> sp., <i>Haemophilus</i> sp.	37-40 ³
40				
45				
50				
55				
60				
65				
70				
75				

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Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5				
<i>bla_{CARB}</i> ⁵	β-lactams	<i>Pseudomonas</i> sp., <i>Enterobacteriaceae</i>	J05162 S46063 M69058 U14749 D86225 D13210 Z18955 AF071555 AF153200 AF030945	
10				
15				
<i>bla_{CTX-M-1}</i> ⁵	β-lactams	<i>Enterobacteriaceae</i>	X92506	
<i>bla_{CTX-M-2}</i> ⁵	β-lactams	<i>Enterobacteriaceae</i>	X92507	
<i>bla_{CMY-2}</i> ⁷	β-lactams	<i>Enterobacteriaceae</i>	X91840	
20				
			AJ007826 AJ011293 AJ011291 Y17716 Y16783 Y16781 Y15130 U77414 S83226 Y15412 X78117	
25				
30				
<i>bla_{IMP}</i> ⁵	β-lactams	<i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i>	AJ223604 S71932 D50438 D29636 X98393 AB010417 D78375	
35				
<i>bla_{PER-1}</i> ⁵	β-lactams	<i>Enterobacteriaceae</i> , <i>Pseudomonadaceae</i>	Z21957	
40				
<i>bla_{PER-2}</i> ⁷	β-lactams	<i>Enterobacteriaceae</i>	X93314	
<i>bla_Z</i> ¹²	β-lactams	<i>Enterococcus</i> sp., <i>Staphylococcus</i> sp.		111 ⁴
<i>mecA</i> ¹²	β-lactams	<i>Staphylococcus</i> sp.		97-98 ³

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Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5 <i>pbp1a</i> ¹³	β-lactams	<i>Streptococcus pneumoniae</i>	M90527 X67872 AB006868 AB006874 X67873 AB006878 AB006875 AB006877 AB006879 AF046237 AF046235 AF026431 AF046232 AF046233 AF046236 X67871 Z49095 AF046234 AB006873 X67866 X67868 AB006870 AB006869 AB006872 X67870 AB006871 X67867 X67869 AB006876 AF046230 AF046238 Z49094	1004-1018, 1648,2056-2064, 2273-2276
10				
15				
20				
25				
30				
35				
40 <i>pbp2b</i> ¹³	β-lactams	<i>Streptococcus pneumoniae</i>	X16022 M25516 M25518 M25515 U20071 U20084 U20082 U20067 U20079 Z22185 U20072 U20083 U20081 M25522 U20075 U20070 U20077 U20068 Z22184 U20069 U20078 M25521 M25525 M25519 Z21981 M25523 M25526 U20076 U20074 M25520 M25517 M25524 Z22230 U20073 U20080	1019-1033
45				
50 <i>pbp2b</i> ¹³	β-lactams	<i>Streptococcus pneumoniae</i>		
55				
60				
65				
70				
75				

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Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5 <i>pbp2x</i> ¹³	β-lactams	<i>Streptococcus pneumoniae</i>	X16367 X65135 AB011204 AB011209 AB011199 AB011200 AB011201 AB011202 AB011198 AB011208 AB011205 AB015852 AB011210 AB015849 AB015850 AB015851 AB015847 AB015846 AB011207 AB015848 Z49096	1034-1048
10				
15				
20				
25				
<i>int</i>	-lactams,	<i>Enterobacteriaceae</i> ,		99-102 ³
30 <i>sul</i>	trimethoprim aminoglycosides, antiseptic, chloramphenicol	<i>Pseudomonads</i>		103-106 ³
<i>ermA</i> ¹⁴	Macrolides, lincosamides, streptogramin B	<i>Staphylococcus</i> sp.		113 ⁴
35 <i>ermB</i> ¹⁴	Macrolides, lincosamides, streptogramin B	<i>Enterobacteriaceae</i> , <i>Staphylococcus</i> sp. <i>Enterococcus</i> sp. <i>Streptococcus</i> sp.		114 ⁴
40 <i>ermC</i> ¹⁴	Macrolides, lincosamides, streptogramin B	<i>Enterobacteriaceae</i> , <i>Staphylococcus</i> sp.		115 ⁴
45 <i>ereA</i> ¹²	Macrolides	<i>Enterobacteriaceae</i> , <i>Staphylococcus</i> sp.	M11277 E01199 AF099140 A15097 X03988	
<i>ereB</i> ¹²	Macrolides	<i>Enterobacteriaceae</i> <i>Staphylococcus</i> sp.		
50 <i>msrA</i> ¹²	Macrolides	<i>Staphylococcus</i> sp.		77-80 ³
<i>mefA</i> , <i>mefE</i> ⁸	Macrolides	<i>Streptococcus</i> sp.	U70055 U83667 D16251 U34344 U36578 J03947 M14039 A15070 E01245 AF110130 AJ238249	
55 <i>mphA</i> ⁸	Macrolides	<i>Enterobacteriaceae</i> , <i>Staphylococcus</i> sp.		
<i>linA/linA'</i> ⁹	Lincosamides	<i>Staphylococcus</i> sp.		
60 <i>linB</i> ¹⁰	Lincosamides	<i>Enterococcus faecium</i>		
<i>vga</i> ¹⁵	Streptogramin	<i>Staphylococcus</i> sp.	M90056 U82085 M36022 M20219 AF015628	89-90 ³
65 <i>vgb</i> ¹⁵	Streptogramin	<i>Staphylococcus</i> sp.		

Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

	Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5	<i>vat</i> ¹⁵ <i>vatB</i> ¹⁵	Streptogramin Streptogramin	<i>Staphylococcus</i> sp. <i>Staphylococcus</i> sp.	L07778 U19459 L38809	87-88 ³
10	<i>sarA</i> ¹⁵ <i>mupA</i> ¹²	Streptogramin Mupirocin	<i>Enterococcus faecium</i> <i>Staphylococcus aureus</i>	L12033 X75439 X59478 X59477	81-82 ³
15	<i>gyrA</i> ¹⁶	Quinolones	Gram-positive and gram-negative bacteria	X95718 X06744 X57174 X16817 X71437 AF065152 AF060881 D32252	1255, 1607-1608, 1764-1776, 2013-2014, 2277-2280
20	<i>parC/grlA</i> ¹⁶	Quinolones	Gram-positive and gram-negative bacteria	AB005036 AF056287 X95717 AF129764 AB017811 AF065152	1777-1785
25	<i>parE/grlB</i> ¹⁶	Quinolones	Gram-positive bacteria	X95717 AF065153 AF058920	
30	<i>norA</i> ¹⁶	Quinolones	<i>Staphylococcus</i> sp.	D90119 M80252 M97169	
35	<i>mexR (nalB)</i> ¹⁶ <i>nfxB</i> ¹⁶ <i>cat</i> ¹²	Quinolones Quinolones Chloramphenicol	<i>Pseudomonas aeruginosa</i> <i>Pseudomonas aeruginosa</i> Gram-positive and gram-negative bacteria	U23763 X65646 M55620 X15100 A24651 M28717 A00568 A00569 X74948 Y00723 A24362 A00569 M93113 M62822 M58516 V01277 X02166 M77169 X53796 J01841 X07848	
40					
45					
50					
55	<i>ppfA</i> -like <i>embB</i> ¹⁷ <i>pncA</i> ¹⁷	Chloramphenicol Ethambutol Pyrazinamide	<i>Mycobacterium tuberculosis</i> <i>Mycobacterium tuberculosis</i>	AF071555 U68480 U59967	
60	<i>rpoB</i> ¹⁷	Rifampin	<i>Mycobacterium tuberculosis</i>	AF055891 AF055892 S71246 L27989 AF055893 AF106077 U02492	
65	<i>inhA</i> ¹⁷	Isoniazid	<i>Mycobacterium tuberculosis</i>		

Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5				
<i>vanA</i> ¹²	Vancomycin	<i>Enterococcus</i> sp.		67-70 ³
<i>vanB</i> ¹²	Vancomycin	<i>Enterococcus</i> sp.		1049-1057
<i>vanCI</i> ¹²	Vancomycin	<i>Enterococcus gallinarum</i>		116 ⁴
10				117 ⁴
<i>vanC2</i> ¹²	Vancomycin	<i>Enterococcus casseliflavus</i>		1058-1059
			U94521	1060-1063
			U94522	
15			U94523	
			U94524	
			U94525	
			L29638	
<i>vanC3</i> ¹²	Vancomycin	<i>Enterococcus flavescens</i>		1064-1066
20			L29639	
<i>vanD</i> ¹⁸	Vancomycin	<i>Enterococcus faecium</i>	U72706	
<i>vanE</i> ¹²	Vancomycin	<i>Enterococcus faecium</i>	AF130997	
<i>tetB</i> ¹⁹	Tetracycline	Gram-negative bacteria	AF136925	
			J01830	
25			AF162223	
			AP000342	
			S83213	
			U81141	
			V00611	
<i>tetM</i> ¹⁹	Tetracycline	Gram-negative and Gram-positive bacteria	X52632	
30			AF116348	
			U50983	
			X92947	
			M211136	
			U08812	
35			X04388	
<i>sul II</i> ²⁰	Sulfonamides	Gram-negative bacteria	M36657	
			AF017389	
			AF017391	
<i>dhfrIa</i> ²⁰	Trimethoprim	Gram-negative bacteria	AJ238350	
40			X17477	
			K00052	
			U09476	
			X00926	
<i>dhfrIb</i> ²⁰	Trimethoprim	Gram-negative bacteria	Z50805	
45			Z50804	
<i>dhfrV</i> ²⁰	Trimethoprim	Gram-negative bacteria	X12868	
<i>dhfrVI</i> ²⁰	Trimethoprim	Gram-negative bacteria	Z86002	
<i>dhfrVII</i> ²⁰	Trimethoprim	Gram-negative bacteria	U31119	
			AF139109	
50			X58425	
<i>dhfrVIII</i> ²⁰	Trimethoprim	Gram-negative bacteria	U10186	
			U09273	
<i>dhfrIX</i> ²⁰	Trimethoprim	Gram-negative bacteria	X57730	
<i>dhfrXII</i> ²⁰	Trimethoprim	Gram-negative bacteria	Z21672	
55			AF175203	
			AF180731	
			M84522	
<i>dhfrXIII</i> ²⁰	Trimethoprim	Gram-negative bacteria	Z50802	
<i>dhfrXV</i> ²⁰	Trimethoprim	Gram-negative bacteria	Z83331	
60			AF170088	
<i>dhfrXVII</i> ²⁰	Trimethoprim	Gram-negative bacteria	AF180469	
			AF169041	

Table 5. Antimicrobial agents resistance genes selected for diagnostic purposes (continued).

Gene	Antimicrobial agent	Bacteria ¹	ACCESSION NO.	SEQ ID NO.
5 <i>dfrA</i> ²⁰	Trimethoprim	<i>Staphylococcus</i> sp.	AF045472 U40259 AF051916 X13290 Y07536 Z16422 Z48233	
10				
15				
20				
25				
30				
35				
40				
45				
50				
55				
60				

1 Bacteria having high incidence for the specified antibiotic resistance gene. The presence of the antibiotic resistance genes in other bacteria is not excluded.

2 Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol. Rev.* 57:138-163.

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4 These SEQ ID NOs. refer to a previous patent (publication WO98/20157).

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6 Nucleotide mutations in *bla*_{SHV}, *bla*_{TEM}, and *bla*_{OXA}, are associated with extended-spectrum β -lactamase or inhibitor-resistant β -lactamase.

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9 Leclerc, R., A., Brisson-Noël, J. Duval, and P. Courvalin. 1991. Phenotypic expression and genetic heterogeneity of lincosamide inactivation in *Staphylococcus* sp. *Antimicrob. Agents. Chemother.* 31:1887-1891.

10 Bozdogan, B., L. Berrezouga, M.-S. Kuo, D. A. Yurek, K. A. Farley, B. J. Stockman, and R. Leclercq. 1999. A new gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. *Antimicrob. Agents. Chemother.* 43:925-929.

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13 Dowson, C. G., T. J. Tracey, and B. G. Spratt. 1994. Origin and molecular epidemiology of penicillin-binding-protein-mediated resistance to β -lactam antibiotics. *Trends Molec. Microbiol.* 2: 361-366.

14 Jensen, L. B., N. Frimodt-Moller, F. M. Aarestrup. 1999. Presence of *erm* gene classes in Gram-positive bacteria of animal and human origin in Denmark. *FEMS Microbiol.* 170:151-158.

15 Thal, L. A., and M. J. Zervos. 1999. Occurrence and epidemiology of resistance to virginimycin and streptogramins. *J. Antimicrob. Chemother.* 43:171-176.

16 Martinez J. L., A. Alonso, J. M. Gomez-Gomez, and F. Baquero. 1998. Quinolone resistance by mutations in chromosomal gyrase genes. Just the tip of the iceberg? *J. Antimicrob. Chemother.* 42:683-688

17 Cockerill III, F.R. 1999. Genetic methods for assessing antimicrobial resistance. *Antimicrob. Agents. Chemother.* 43:199-212.

18 Casadewall, B. and P. Courvalin. 1999 Characterization of the *vanD* glycopeptide resistance gene cluster from *Enterococcus faecium* BM 4339. *J. Bacteriol.* 181:3644-3648.

19 Roberts, M.C. 1999. Genetic mobility and distribution of tetracycline resistance determinants. *Ciba Found. Symp.* 207:206-222.

20 Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld. 1995. Trimethoprim and sulfonamide resistance. *Antimicrob. Agent Chemother.* 39:279-289.

Table 6. List of bacterial toxins selected for diagnostic purposes.

	Organism	Toxin	Accession number
5	<i>Actinobacillus actinomycetemcomitans</i>	Cytolethal distending toxin (<i>cdtA</i> , <i>cdtB</i> , <i>cdtC</i>)	AF006830
		Leukotoxin (<i>ltxA</i>)	M27399
	<i>Actinomyces pyogenes</i>	Hemolysin (pyolysin)	U84782
10	<i>Aeromonas hydrophila</i>	Aerolysin (<i>aerA</i>)	M16495
		Haemolysin (<i>hlyA</i>)	U81555
		Cytotoxic enterotoxin (<i>alt</i>)	L77573
15	<i>Bacillus anthracis</i>	Anthrax toxin (<i>cya</i>)	M23179
	<i>Bacillus cereus</i>	Enterotoxin (<i>bceT</i>)	D17312
			AF192766, AF192767
		Enterotoxic hemolysin BL	AJ237785
20	<i>Bacillus mycoides</i>	Non-haemolytic enterotoxins A,B and C (<i>nhe</i>)	Y19005
	<i>Bacillus pseudomycoides</i>	Hemolytic enterotoxin HBL	AJ243150 to AJ243153
	<i>Bacteroides fragilis</i>	Hemolytic enterotoxin HBL	AJ243154 to AJ243156
		Enterotoxin (<i>bftP</i>)	U67735
25		Matrix metalloprotease/enterotoxin (fragilysin)	S75941, AF038459
		Metalloprotease toxin-2	U90931
			AF081785
30	<i>Bordetella bronchiseptica</i>	Metalloprotease toxin-3	AF056297
		Adenylate cyclase hemolysin (<i>cyaA</i>)	Z37112, U22953
		Dermonecrotic toxin (<i>dnt</i>)	U59687
35	<i>Bordetella pertussis</i>	Pertussis toxin (S1 subunit, <i>tox</i>)	AB020025
			AJ006151
			AJ006153
			AJ006155
			AJ006157
40			AJ006159
			AJ007363
			M14378, M16494
			AJ007364
			M13223
45			X16347
		Adenyl cyclase (<i>cya</i>)	18323
		Dermonecrotic toxin (<i>dnt</i>)	U10527
50	<i>Campylobacter jejuni</i>	Cytolethal distending toxin (<i>cdtA</i> , <i>cdtB</i> , <i>cdtC</i>)	U51121
	<i>Citrobacter freundii</i>	Shiga-like toxin (<i>slt-IIcA</i>)	X67514, S53206
	<i>Clostridium botulinum</i>	Botulism toxin (BoNT) (A,B,E and F serotypes are neurotoxic for humans; the other serotypes have not been considered)	X52066, X52088
			X73423
			M30196
55			X70814
			X70819
			X71343
			Z11934
			X70817
			M81186
60			X70818
			X70815
			X62089
			X62683
65			S76749
			X81714
			X70816

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Table 6. List of bacterial toxins selected for diagnostic purposes (continued).

	Organism	Toxin	Accession number
5	<i>Clostridium botulinum</i> (continued)		X70820 X70281 L35496 M92906
10	<i>Clostridium difficile</i>	A toxin (enterotoxin) (<i>tcdA</i>) (<i>cdtA</i>)	AB012304 AF053400 Y12616 X51797 X17194 M30307
15		B toxin (cytotoxin) (<i>toxB</i>) (<i>cdtB</i>)	Z23277 X53138
	<i>Clostridium perfringens</i>	Alpha (phospholipase C) (<i>cpa</i>)	L43545 L43546 L43547 L43548 X13608 X17300 D10248
20			
25		Beta (dermonecrotic protein) (<i>cpb</i>)	L13198 X83275 L77965
30		Enterotoxin (<i>cpe</i>)	AJ000766 M98037 X81849 X71844 Y16009
35		Enterotoxin pseudogene (not expressed)	AF037328 AF037329 AF037330
40		Epsilon toxin (<i>etxD</i>)	M80837 M95206 X60694
		Iota (Ia and Ib)	X73562
45		Lambda (metalloprotease)	D45904
	<i>Clostridium sordellii</i>	Theta (perfringolysin O)	M36704
	<i>Clostridium tetani</i>	Cytotoxin L	X82638
50		Tetanospasmin	X06214 X04436 X00703
	<i>Corynebacterium diphtheriae</i>	Diphtheriae toxin	
	<i>Corynebacterium pseudotuberculosis</i>	Phospholipase C	A21336
55	<i>Eikenella corrodens</i>	lysine decarboxylase (<i>cadA</i>)	U89166
	<i>Enterobacter cloacae</i>	Shiga-like toxin II	Z50754, U33502
	<i>Enterococcus faecalis</i>	Cytolysin B (<i>cylB</i>)	M38052
	<i>Escherichia coli</i> (EHEC)	Hemolysin toxin (<i>hlyA</i> and <i>ehxA</i>)	AF043471 X94129 X79839 X86087 AB011549 AF074613
60			

Table 6. List of bacterial toxins selected for diagnostic purposes (continued).

	Organism	Toxin	Accession number
5	<i>Escherichia coli</i> (EHEC)	Shiga-like (Vero cytotoxin) (<i>stx</i>)	X81418, M36727 M14107, E03962 M10133, E03959 M12863, X07865 X81417, Y10775 X81416, Z50754 X81415, X67515 Z36900, AF043627 L11078, M19473 L04539, M17358 L11079, M19437 X65949, M24352 M21534, X07903 M29153, Z36899 Z37725 Z36901 X61283 AB017524 U72191 X61283 M17874 M17873 J01605 AB011677
10			
15			
20			
25	<i>Escherichia coli</i> (ETEC)	Enterotoxin (heat-labile) (<i>eltB</i>)	L11241 M58746 M29255 V00612 J01831 U03293 U04208 U89305
30		Enterotoxin (heat-stable) (<i>astA</i>) (<i>estA1</i>)	U42629
35	<i>Escherichia coli</i> (other)	Cytotoxin (heat-stable) (<i>stx</i>)	U47048 AF056581 U53215 U07145 U80067 U80068 AF077938 AF077939 AF077940 AF077941 AF057703 X15127 M24199 X60035 U25452 U25443 U25446 U25449 X57775, Z28388 X51512 X52478 M30186 X14956 AF060869
40		Cytotoxic necrotizing factor 1 (<i>cnf1</i>)	
45	<i>Haemophilus ducreyi</i> <i>Helicobacter pylori</i>	Microcin 24 (<i>mtfS</i>) Autotransporter enterotoxin (<i>Pet</i>) (cytotoxin) Cytotoxin distending toxin (<i>cdtA</i> , <i>cdtB</i> , <i>cdtC</i>) Vacuolating toxin (<i>vacA</i>)	
50	<i>Legionella pneumophila</i> <i>Listeria monocytogenes</i>	Structural toxin protein (<i>rtxA</i>) Listeriolysin O (<i>lisA</i> , <i>hlyA</i>)	
55			
60	<i>Pasteurella multocida</i>	Mitogenic toxin (dermonecrotic toxin)	
65	<i>Proteus mirabilis</i> <i>Pseudomonas aeruginosa</i> <i>Salmonella typhimurium</i>	Hemolysin (<i>hpmA</i>) Cytotoxin (Enterotoxin A) Calmodulin-sensitive adenylate cyclase toxin (<i>cya</i>)	
		Cytolysin (salmolysin) (<i>shyA</i>)	U03842
		Enterotoxin (<i>stn</i>)	L16014

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Table 6. List of bacterial toxins selected for diagnostic purposes (continued).

	Organism	Toxin	Accession number
5	<i>Serratia marcescens</i> <i>Shigella dysenteriae</i> type 1	Hemolysin (<i>shlA</i>) Shiga toxin (<i>stxA</i> and <i>stxB</i>)	M22618 X07903, M32511 M19437 M24352, M21947
10	<i>Shigella flexneri</i>	ShET2 enterotoxin (<i>senA</i>) Enterotoxin 1 (<i>set1A</i> and <i>set1B</i>)	Z54211 Z47381 U35656
15	<i>Shigella sonnei</i> <i>Sphingomonas paucimobilis</i> <i>Staphylococcus aureus</i>	Hemolysin E (<i>hlyE</i> , <i>clyA</i> , <i>sheA</i>) Shiga toxin (<i>stxA</i> and <i>stxB</i>) Beta-hemolysin (<i>hlyA</i>) Gamma-hemolysin (<i>hlg2</i>)	AF200955 AJ132761 L01270 D42143 L01055
20		Enterotoxin Enterotoxin A (<i>sea</i>)	U93688 L22565, L22566 M18970
25		Enterotoxin B Enterotoxin C1 (<i>entC1</i>)	M11118 X05815
30		Enterotoxin C2 (<i>entC2</i>) Enterotoxin C3 (<i>entC3</i>) Enterotoxin D (<i>sed</i>)	P34071 X51661 M94872
35		Enterotoxin E Enterotoxin G (<i>seg</i>) Enterotoxin H (<i>seh</i>)	M21319 AF064773 U11702
40		Enterotoxin I (<i>sei</i>) Enterotoxin J	AF064774 AF053140
45		Exfoliative toxin A (ETA, Epidermolytic toxin A) Exfoliative toxin B (ETB)	M17347 M17357 L25372, M20371 M17348, M13775
50		Leukocidin R (F and S component, <i>lukF</i> and <i>lukS</i> ; Hemolysin B and C)	X64389, S53213 X72700 L01055
55		Toxic shock syndrome toxin 1 (TSST-1, alpha toxin, alpha hemolysin)	X01645 M90536 J02615 U93688
60	<i>Staphylococcus epidermidis</i> <i>Staphylococcus intermedius</i>	Delta toxin (<i>hld</i>) Enterotoxin 1	AF068634 U91526
65	<i>Streptococcus pneumoniae</i>	Leukocidin R (F and S component, <i>lukF</i> and <i>lukS</i> ; synergohymenotropic toxin) Pneumolysin	X79188 X52474

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Table 6. List of bacterial toxins selected for diagnostic purposes (continued).

	Organism	Toxin	Accession number
5	<i>Streptococcus pyogenes</i>	<i>Streptococcus</i> pyrogenic exotoxin A (<i>speA</i>)	X61553 to X61573 X03929 U40453, M19350
		Pyrogenic exotoxin B (<i>speB</i>) M86905, M35110	U63134
10	<i>Vibrio cholerae</i>	Cholerae toxin (<i>ctxA</i> and <i>ctxB</i> subunits)	X00171 X76390 X58786 X58785, S55782 D30052 D30053 K02679 AF175708
15			
20		Accessory cholera enterotoxin (<i>ace</i>)	Z22569, AF175708
		Heat-stable enterotoxin (<i>sto</i>)	X74108, M85198 M97591, L03220
25	<i>Vibrio parahaemolyticus</i>	<i>Zonula occludens</i> toxin (<i>zot</i>)	M83563, AF175708
		Thermostable direct hemolysin (<i>tdh</i>)	S67841
	<i>Vibrio vulnificus</i>	Cytolysin (<i>vvhA</i>)	M34670
	<i>Yersinia enterocolitica</i>	Heat-stable enterotoxin (<i>yst</i>)	U09235, X65999
30		Heat-stable enterotoxin type B (<i>ystB</i>)	D88145
		Heat-stable enterotoxin type C (<i>ystC</i>)	D63578
	<i>Yersinia kristensenii</i>	Enterotoxin X69218	
	<i>Yersinia pestis</i>	Toxin	X92727
35			

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	SourceGene*
5	1 <i>Acinetobacter baumannii</i>	This patent <i>tuf</i>
	2 <i>Actinomyces meyeri</i>	This patent <i>tuf</i>
	3 <i>Aerococcus viridans</i>	This patent <i>tuf</i>
	4 <i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i>	This patent <i>tuf</i>
	5 <i>Anaerorhabdus furcosus</i>	This patent <i>tuf</i>
10	6 <i>Bacillus anthracis</i>	This patent <i>tuf</i>
	7 <i>Bacillus cereus</i>	This patent <i>tuf</i>
	8 <i>Bacteroides distasonis</i>	This patent <i>tuf</i>
	9 <i>Enterococcus casseliflavus</i>	This patent <i>tuf</i>
	10 <i>Staphylococcus saprophyticus</i>	This patent <i>tuf</i>
15	11 <i>Bacteroides ovatus</i>	This patent <i>tuf</i>
	12 <i>Bartonella henselae</i>	This patent <i>tuf</i>
	13 <i>Bifidobacterium adolescentis</i>	This patent <i>tuf</i>
	14 <i>Bifidobacterium dentium</i>	This patent <i>tuf</i>
	15 <i>Brucella abortus</i>	This patent <i>tuf</i>
20	16 <i>Burkholderia cepacia</i>	This patent <i>tuf</i>
	17 <i>Cedecea davisae</i>	This patent <i>tuf</i>
	18 <i>Cedecea neteri</i>	This patent <i>tuf</i>
	19 <i>Cedecea lapagei</i>	This patent <i>tuf</i>
	20 <i>Chlamydia pneumoniae</i>	This patent <i>tuf</i>
25	21 <i>Chlamydia psittaci</i>	This patent <i>tuf</i>
	22 <i>Chlamydia trachomatis</i>	This patent <i>tuf</i>
	23 <i>Chryseobacterium meningosepticum</i>	This patent <i>tuf</i>
	24 <i>Citrobacter amalonaticus</i>	This patent <i>tuf</i>
	25 <i>Citrobacter braakii</i>	This patent <i>tuf</i>
30	26 <i>Citrobacter koseri</i>	This patent <i>tuf</i>
	27 <i>Citrobacter farmeri</i>	This patent <i>tuf</i>
	28 <i>Citrobacter freundii</i>	This patent <i>tuf</i>
	29 <i>Citrobacter sedlakii</i>	This patent <i>tuf</i>
	30 <i>Citrobacter werkmanii</i>	This patent <i>tuf</i>
35	31 <i>Citrobacter youngae</i>	This patent <i>tuf</i>
	32 <i>Clostridium perfringens</i>	This patent <i>tuf</i>
	33 <i>Comamonas acidovorans</i>	This patent <i>tuf</i>
	34 <i>Corynebacterium bovis</i>	This patent <i>tuf</i>
	35 <i>Corynebacterium cervicis</i>	This patent <i>tuf</i>
40	36 <i>Corynebacterium flavesces</i>	This patent <i>tuf</i>
	37 <i>Corynebacterium kutscheri</i>	This patent <i>tuf</i>
	38 <i>Corynebacterium minutissimum</i>	This patent <i>tuf</i>
	39 <i>Corynebacterium mycetoides</i>	This patent <i>tuf</i>
	40 <i>Corynebacterium pseudogenitalium</i>	This patent <i>tuf</i>
45	41 <i>Corynebacterium renale</i>	This patent <i>tuf</i>
	42 <i>Corynebacterium ulcerans</i>	This patent <i>tuf</i>
	43 <i>Corynebacterium urealyticum</i>	This patent <i>tuf</i>
	44 <i>Corynebacterium xerosis</i>	This patent <i>tuf</i>
	45 <i>Coxiella burnetii</i>	This patent <i>tuf</i>
50	46 <i>Edwardsiella hoshinae</i>	This patent <i>tuf</i>
	47 <i>Edwardsiella tarda</i>	This patent <i>tuf</i>
	48 <i>Eikenella corrodens</i>	This patent <i>tuf</i>
	49 <i>Enterobacter aerogenes</i>	This patent <i>tuf</i>
	50 <i>Enterobacter agglomerans</i>	This patent <i>tuf</i>
55	51 <i>Enterobacter amnigenus</i>	This patent <i>tuf</i>
	52 <i>Enterobacter asburiae</i>	This patent <i>tuf</i>
	53 <i>Enterobacter cancerogenus</i>	This patent <i>tuf</i>
	54 <i>Enterobacter cloacae</i>	This patent <i>tuf</i>
	55 <i>Enterobacter gergoviae</i>	This patent <i>tuf</i>
60	56 <i>Enterobacter hormaechei</i>	This patent <i>tuf</i>
	57 <i>Enterobacter sakazakii</i>	This patent <i>tuf</i>
	58 <i>Enterococcus casseliflavus</i>	This patent <i>tuf</i>
	59 <i>Enterococcus cecorum</i>	This patent <i>tuf</i>
	60 <i>Enterococcus dispar</i>	This patent <i>tuf</i>
65	61 <i>Enterococcus durans</i>	This patent <i>tuf</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	62 <i>Enterococcus faecalis</i>	This patent	tuf
	63 <i>Enterococcus faecalis</i>	This patent	tuf
	64 <i>Enterococcus faecium</i>	This patent	tuf
	65 <i>Enterococcus flavescens</i>	This patent	tuf
	66 <i>Enterococcus gallinarum</i>	This patent	tuf
10	67 <i>Enterococcus hirae</i>	This patent	tuf
	68 <i>Enterococcus mundtii</i>	This patent	tuf
	69 <i>Enterococcus pseudoavium</i>	This patent	tuf
	70 <i>Enterococcus raffinosus</i>	This patent	tuf
	71 <i>Enterococcus saccharolyticus</i>	This patent	tuf
15	72 <i>Enterococcus solitarius</i>	This patent	tuf
	73 <i>Enterococcus casseliflavus</i>	This patent	tuf (C)
	74 <i>Staphylococcus saprophyticus</i>	This patent	unknown
	75 <i>Enterococcus flavescens</i>	This patent	tuf (C)
	76 <i>Enterococcus gallinarum</i>	This patent	tuf (C)
20	77 <i>Ehrlichia canis</i>	This patent	tuf
	78 <i>Escherichia coli</i>	This patent	tuf
	79 <i>Escherichia fergusonii</i>	This patent	tuf
	80 <i>Escherichia hermannii</i>	This patent	tuf
	81 <i>Escherichia vulneris</i>	This patent	tuf
25	82 <i>Eubacterium lentum</i>	This patent	tuf
	83 <i>Eubacterium nodatum</i>	This patent	tuf
	84 <i>Ewingella americana</i>	This patent	tuf
	85 <i>Francisella tularensis</i>	This patent	tuf
	86 <i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>	This patent	tuf
30	87 <i>Gemella haemolysans</i>	This patent	tuf
	88 <i>Gemella morbillorum</i>	This patent	tuf
	89 <i>Haemophilus actinomycetemcomitans</i>	This patent	tuf
	90 <i>Haemophilus aphrophilus</i>	This patent	tuf
	91 <i>Haemophilus ducreyi</i>	This patent	tuf
35	92 <i>Haemophilus haemolyticus</i>	This patent	tuf
	93 <i>Haemophilus parahaemolyticus</i>	This patent	tuf
	94 <i>Haemophilus parainfluenzae</i>	This patent	tuf
	95 <i>Haemophilus paraphrophilus</i>	This patent	tuf
	96 <i>Haemophilus segnis</i>	This patent	tuf
40	97 <i>Hafnia alvei</i>	This patent	tuf
	98 <i>Kingella kingae</i>	This patent	tuf
	99 <i>Klebsiella ornithinolytica</i>	This patent	tuf
	100 <i>Klebsiella oxytoca</i>	This patent	tuf
	101 <i>Klebsiella planticola</i>	This patent	tuf
45	102 <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	This patent	tuf
	103 <i>Klebsiella pneumoniae pneumoniae</i>	This patent	tuf
	104 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>	This patent	tuf
	105 <i>Kluyvera ascorbata</i>	This patent	tuf
	106 <i>Kluyvera cryocrescens</i>	This patent	tuf
50	107 <i>Kluyvera georgiana</i>	This patent	tuf
	108 <i>Lactobacillus casei</i> subsp. <i>casei</i>	This patent	tuf
	109 <i>Lactococcus lactis</i> subsp. <i>lactis</i>	This patent	tuf
	110 <i>Leclercia adecarboxylata</i>	This patent	tuf
	111 <i>Legionella micdadei</i>	This patent	tuf
55	112 <i>Legionella pneumophila</i> subsp. <i>pneumophila</i>	This patent	tuf
	113 <i>Leminorella grimontii</i>	This patent	tuf
	114 <i>Leminorella richardii</i>	This patent	tuf
	115 <i>Leptospira interrogans</i>	This patent	tuf
	116 <i>Megamonas hypermegale</i>	This patent	tuf
60	117 <i>Mitsuokella multacidus</i>	This patent	tuf
	118 <i>Mobiluncus curtisii</i> subsp. <i>holmesii</i>	This patent	tuf
	119 <i>Moellerella wisconsensis</i>	This patent	tuf
	120 <i>Moraxella catarrhalis</i>	This patent	tuf
	121 <i>Morganella morganii</i> subsp. <i>morganii</i>	This patent	tuf
65	122 <i>Mycobacterium tuberculosis</i>	This patent	tuf

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.		Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	123	<i>Neisseria cinerea</i>	This patent	<i>tuf</i>
	124	<i>Neisseria elongata</i> subsp. <i>elongata</i>	This patent	<i>tuf</i>
	125	<i>Neisseria flavescens</i>	This patent	<i>tuf</i>
	126	<i>Neisseria gonorrhoeae</i>	This patent	<i>tuf</i>
	127	<i>Neisseria lactamica</i>	This patent	<i>tuf</i>
10	128	<i>Neisseria meningitidis</i>	This patent	<i>tuf</i>
	129	<i>Neisseria mucosa</i>	This patent	<i>tuf</i>
	130	<i>Neisseria sicca</i>	This patent	<i>tuf</i>
	131	<i>Neisseria subflava</i>	This patent	<i>tuf</i>
	132	<i>Neisseria weaveri</i>	This patent	<i>tuf</i>
15	133	<i>Ochrobactrum anthropi</i>	This patent	<i>tuf</i>
	134	<i>Pantoea agglomerans</i>	This patent	<i>tuf</i>
	135	<i>Pantoea dispersa</i>	This patent	<i>tuf</i>
	136	<i>Pasteurella multocida</i>	This patent	<i>tuf</i>
	137	<i>Peptostreptococcus anaerobius</i>	This patent	<i>tuf</i>
20	138	<i>Peptostreptococcus asaccharolyticus</i>	This patent	<i>tuf</i>
	139	<i>Peptostreptococcus prevotii</i>	This patent	<i>tuf</i>
	140	<i>Porphyromonas asaccharolytica</i>	This patent	<i>tuf</i>
	141	<i>Porphyromonas gingivalis</i>	This patent	<i>tuf</i>
	142	<i>Pragia fontium</i>	This patent	<i>tuf</i>
25	143	<i>Prevotella melaninogenica</i>	This patent	<i>tuf</i>
	144	<i>Prevotella oralis</i>	This patent	<i>tuf</i>
	145	<i>Propionibacterium acnes</i>	This patent	<i>tuf</i>
	146	<i>Proteus mirabilis</i>	This patent	<i>tuf</i>
	147	<i>Proteus penneri</i>	This patent	<i>tuf</i>
30	148	<i>Proteus vulgaris</i>	This patent	<i>tuf</i>
	149	<i>Providencia alcalifaciens</i>	This patent	<i>tuf</i>
	150	<i>Providencia rettgeri</i>	This patent	<i>tuf</i>
	151	<i>Providencia rustigianii</i>	This patent	<i>tuf</i>
	152	<i>Providencia stuartii</i>	This patent	<i>tuf</i>
35	153	<i>Pseudomonas aeruginosa</i>	This patent	<i>tuf</i>
	154	<i>Pseudomonas fluorescens</i>	This patent	<i>tuf</i>
	155	<i>Pseudomonas stutzeri</i>	This patent	<i>tuf</i>
	156	<i>Psychrobacter phenylpyruvicum</i>	This patent	<i>tuf</i>
	157	<i>Rahnella aquatilis</i>	This patent	<i>tuf</i>
40	158	<i>Salmonella choleraesuis</i> subsp. <i>arizonae</i>	This patent	<i>tuf</i>
	159	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Choleraesuis	This patent	<i>tuf</i>
	160	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	This patent	<i>tuf</i>
	161	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Heidelberg	This patent	<i>tuf</i>
	162	<i>Salmonella choleraesuis</i> subsp. <i>houtenae</i>	This patent	<i>tuf</i>
45	163	<i>Salmonella choleraesuis</i> subsp. <i>indica</i>	This patent	<i>tuf</i>
	164	<i>Salmonella choleraesuis</i> subsp. <i>salamae</i>	This patent	<i>tuf</i>
	165	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	This patent	<i>tuf</i>
	166	<i>Serratia fonticola</i>	This patent	<i>tuf</i>
	167	<i>Serratia liquefaciens</i>	This patent	<i>tuf</i>
50	168	<i>Serratia marcescens</i>	This patent	<i>tuf</i>
	169	<i>Serratia odorifera</i>	This patent	<i>tuf</i>
	170	<i>Serratia phymuthica</i>	This patent	<i>tuf</i>
	171	<i>Serratia rubidaea</i>	This patent	<i>tuf</i>
	172	<i>Shigella boydii</i>	This patent	<i>tuf</i>
55	173	<i>Shigella dysenteriae</i>	This patent	<i>tuf</i>
	174	<i>Shigella flexneri</i>	This patent	<i>tuf</i>
	175	<i>Shigella sonnei</i>	This patent	<i>tuf</i>
	176	<i>Staphylococcus aureus</i>	This patent	<i>tuf</i>
	177	<i>Staphylococcus aureus</i>	This patent	<i>tuf</i>
60	178	<i>Staphylococcus aureus</i>	This patent	<i>tuf</i>
	179	<i>Staphylococcus aureus</i>	This patent	<i>tuf</i>
	180	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	This patent	<i>tuf</i>
	181	<i>Staphylococcus auricularis</i>	This patent	<i>tuf</i>
	182	<i>Staphylococcus capitis</i> subsp. <i>capitis</i>	This patent	<i>tuf</i>

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	183 <i>Macrococcus caseolyticus</i>	This patent	<i>tuf</i>
	184 <i>Staphylococcus cohnii</i> subsp. <i>cohnii</i>	This patent	<i>tuf</i>
	185 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
	186 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	187 <i>Staphylococcus warneri</i>	This patent	<i>tuf</i>
10	188 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	189 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	190 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	191 <i>Staphylococcus hominis</i> subsp. <i>hominis</i>	This patent	<i>tuf</i>
15	192 <i>Staphylococcus warneri</i>	This patent	<i>tuf</i>
	193 <i>Staphylococcus hominis</i>	This patent	<i>tuf</i>
	194 <i>Staphylococcus hominis</i>	This patent	<i>tuf</i>
	195 <i>Staphylococcus hominis</i>	This patent	<i>tuf</i>
	196 <i>Staphylococcus hominis</i>	This patent	<i>tuf</i>
20	197 <i>Staphylococcus lugdunensis</i>	This patent	<i>tuf</i>
	198 <i>Staphylococcus saprophyticus</i>	This patent	<i>tuf</i>
	199 <i>Staphylococcus saprophyticus</i>	This patent	<i>tuf</i>
	200 <i>Staphylococcus saprophyticus</i>	This patent	<i>tuf</i>
	201 <i>Staphylococcus sciuri</i> subsp. <i>sciuri</i>	This patent	<i>tuf</i>
	202 <i>Staphylococcus warneri</i>	This patent	<i>tuf</i>
25	203 <i>Staphylococcus warneri</i>	This patent	<i>tuf</i>
	204 <i>Bifidobacterium longum</i>	This patent	<i>tuf</i>
	205 <i>Stenotrophomonas maltophilia</i>	This patent	<i>tuf</i>
	206 <i>Streptococcus acidominimus</i>	This patent	<i>tuf</i>
	207 <i>Streptococcus agalactiae</i>	This patent	<i>tuf</i>
30	208 <i>Streptococcus agalactiae</i>	This patent	<i>tuf</i>
	209 <i>Streptococcus agalactiae</i>	This patent	<i>tuf</i>
	210 <i>Streptococcus agalactiae</i>	This patent	<i>tuf</i>
	211 <i>Streptococcus anginosus</i>	This patent	<i>tuf</i>
35	212 <i>Streptococcus bovis</i>	This patent	<i>tuf</i>
	213 <i>Streptococcus anginosus</i>	This patent	<i>tuf</i>
	214 <i>Streptococcus cricetus</i>	This patent	<i>tuf</i>
	215 <i>Streptococcus cristatus</i>	This patent	<i>tuf</i>
	216 <i>Streptococcus downei</i>	This patent	<i>tuf</i>
40	217 <i>Streptococcus dysgalactiae</i>	This patent	<i>tuf</i>
	218 <i>Streptococcus equi</i> subsp. <i>equi</i>	This patent	<i>tuf</i>
	219 <i>Streptococcus ferus</i>	This patent	<i>tuf</i>
	220 <i>Streptococcus gordonii</i>	This patent	<i>tuf</i>
	221 <i>Streptococcus anginosus</i>	This patent	<i>tuf</i>
45	222 <i>Streptococcus macacae</i>	This patent	<i>tuf</i>
	223 <i>Streptococcus gordonii</i>	This patent	<i>tuf</i>
	224 <i>Streptococcus mutans</i>	This patent	<i>tuf</i>
	225 <i>Streptococcus parasanguinis</i>	This patent	<i>tuf</i>
	226 <i>Streptococcus rattus</i>	This patent	<i>tuf</i>
50	227 <i>Streptococcus sanguinis</i>	This patent	<i>tuf</i>
	228 <i>Streptococcus sobrinus</i>	This patent	<i>tuf</i>
	229 <i>Streptococcus suis</i>	This patent	<i>tuf</i>
	230 <i>Streptococcus uberis</i>	This patent	<i>tuf</i>
	231 <i>Streptococcus vestibularis</i>	This patent	<i>tuf</i>
55	232 <i>Tatumella ptyseos</i>	This patent	<i>tuf</i>
	233 <i>Trabulsiella guamensis</i>	This patent	<i>tuf</i>
	234 <i>Veillonella parvula</i>	This patent	<i>tuf</i>
	235 <i>Yersinia enterocolitica</i>	This patent	<i>tuf</i>
	236 <i>Yersinia frederiksenii</i>	This patent	<i>tuf</i>
60	237 <i>Yersinia intermedia</i>	This patent	<i>tuf</i>
	238 <i>Yersinia pestis</i>	This patent	<i>tuf</i>
	239 <i>Yersinia pseudotuberculosis</i>	This patent	<i>tuf</i>
	240 <i>Yersinia rohdei</i>	This patent	<i>tuf</i>
	241 <i>Yokenella regensburgei</i>	This patent	<i>tuf</i>
65	242 <i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i>	This patent	<i>atpD</i>
	243 <i>Acinetobacter baumannii</i>	This patent	<i>atpD</i>
	244 <i>Acinetobacter lwoffii</i>	This patent	<i>atpD</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	245 <i>Staphylococcus saprophyticus</i>	This patent	<i>atpD</i>
	246 <i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	This patent	<i>atpD</i>
	247 <i>Bacillus anthracis</i>	This patent	<i>atpD</i>
	248 <i>Bacillus cereus</i>	This patent	<i>atpD</i>
	249 <i>Bacteroides distasonis</i>	This patent	<i>atpD</i>
10	250 <i>Bacteroides ovatus</i>	This patent	<i>atpD</i>
	251 <i>Leclercia adecarboxylata</i>	This patent	<i>atpD</i>
	252 <i>Stenotrophomonas maltophilia</i>	This patent	<i>atpD</i>
	253 <i>Bartonella henselae</i>	This patent	<i>atpD</i>
	254 <i>Bifidobacterium adolescentis</i>	This patent	<i>atpD</i>
15	255 <i>Brucella abortus</i>	This patent	<i>atpD</i>
	256 <i>Cedecea davisae</i>	This patent	<i>atpD</i>
	257 <i>Cedecea lapagei</i>	This patent	<i>atpD</i>
	258 <i>Cedecea neteri</i>	This patent	<i>atpD</i>
	259 <i>Chryseobacterium meningosepticum</i>	This patent	<i>atpD</i>
20	260 <i>Citrobacter amalonaticus</i>	This patent	<i>atpD</i>
	261 <i>Citrobacter braakii</i>	This patent	<i>atpD</i>
	262 <i>Citrobacter koseri</i>	This patent	<i>atpD</i>
	263 <i>Citrobacter farmeri</i>	This patent	<i>atpD</i>
	264 <i>Citrobacter freundii</i>	This patent	<i>atpD</i>
25	265 <i>Citrobacter koseri</i>	This patent	<i>atpD</i>
	266 <i>Citrobacter sedlakii</i>	This patent	<i>atpD</i>
	267 <i>Citrobacter werkmanii</i>	This patent	<i>atpD</i>
	268 <i>Citrobacter youngae</i>	This patent	<i>atpD</i>
	269 <i>Clostridium innocuum</i>	This patent	<i>atpD</i>
30	270 <i>Clostridium perfringens</i>	This patent	<i>atpD</i>
	272 <i>Corynebacterium diphtheriae</i>	This patent	<i>atpD</i>
	273 <i>Corynebacterium pseudodiphtheriticum</i>	This patent	<i>atpD</i>
	274 <i>Corynebacterium ulcerans</i>	This patent	<i>atpD</i>
	275 <i>Corynebacterium urealyticum</i>	This patent	<i>atpD</i>
35	276 <i>Coxiella burnetii</i>	This patent	<i>atpD</i>
	277 <i>Edwardsiella hoshinae</i>	This patent	<i>atpD</i>
	278 <i>Edwardsiella tarda</i>	This patent	<i>atpD</i>
	279 <i>Eikenella corrodens</i>	This patent	<i>atpD</i>
	280 <i>Enterobacter agglomerans</i>	This patent	<i>atpD</i>
40	281 <i>Enterobacter amnigenus</i>	This patent	<i>atpD</i>
	282 <i>Enterobacter asburiae</i>	This patent	<i>atpD</i>
	283 <i>Enterobacter cancerogenus</i>	This patent	<i>atpD</i>
	284 <i>Enterobacter cloacae</i>	This patent	<i>atpD</i>
	285 <i>Enterobacter gergoviae</i>	This patent	<i>atpD</i>
45	286 <i>Enterobacter hormaechei</i>	This patent	<i>atpD</i>
	287 <i>Enterobacter sakazakii</i>	This patent	<i>atpD</i>
	288 <i>Enterococcus avium</i>	This patent	<i>atpD</i>
	289 <i>Enterococcus casseliflavus</i>	This patent	<i>atpD</i>
	290 <i>Enterococcus durans</i>	This patent	<i>atpD</i>
50	291 <i>Enterococcus faecalis</i>	This patent	<i>atpD</i>
	292 <i>Enterococcus faecium</i>	This patent	<i>atpD</i>
	293 <i>Enterococcus gallinarum</i>	This patent	<i>atpD</i>
	294 <i>Enterococcus saccharolyticus</i>	This patent	<i>atpD</i>
	295 <i>Escherichia fergusonii</i>	This patent	<i>atpD</i>
55	296 <i>Escherichia hermannii</i>	This patent	<i>atpD</i>
	297 <i>Escherichia vulneris</i>	This patent	<i>atpD</i>
	298 <i>Eubacterium lentum</i>	This patent	<i>atpD</i>
	299 <i>Ewingella americana</i>	This patent	<i>atpD</i>
	300 <i>Francisella tularensis</i>	This patent	<i>atpD</i>
60	301 <i>Fusobacterium gonidiaformans</i>	This patent	<i>atpD</i>
	302 <i>Fusobacterium necrophorum</i> subsp. <i>necrophorum</i>	This patent	<i>atpD</i>
	303 <i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>	This patent	<i>atpD</i>
	304 <i>Gardnerella vaginalis</i>	This patent	<i>atpD</i>
	305 <i>Gemella haemolysans</i>	This patent	<i>atpD</i>
65	306 <i>Gemella morbillorum</i>	This patent	<i>atpD</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	307	<i>Haemophilus ducreyi</i>	This patent	<i>atpD</i>
	308	<i>Haemophilus haemolyticus</i>	This patent	<i>atpD</i>
	309	<i>Haemophilus parahaemolyticus</i>	This patent	<i>atpD</i>
	310	<i>Haemophilus parainfluenzae</i>	This patent	<i>atpD</i>
	311	<i>Hafnia alvei</i>	This patent	<i>atpD</i>
10	312	<i>Kingella kingae</i>	This patent	<i>atpD</i>
	313	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	This patent	<i>atpD</i>
	314	<i>Klebsiella ornithinolytica</i>	This patent	<i>atpD</i>
	315	<i>Klebsiella oxytoca</i>	This patent	<i>atpD</i>
15	316	<i>Klebsiella planticola</i>	This patent	<i>atpD</i>
	317	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	<i>atpD</i>
	318	<i>Kluyvera ascorbata</i>	This patent	<i>atpD</i>
	319	<i>Kluyvera cryocrescens</i>	This patent	<i>atpD</i>
	320	<i>Kluyvera georgiana</i>	This patent	<i>atpD</i>
20	321	<i>Lactobacillus acidophilus</i>	This patent	<i>atpD</i>
	322	<i>Legionella pneumophila</i> subsp. <i>pneumophila</i>	This patent	<i>atpD</i>
	323	<i>Leminorella grimontii</i>	This patent	<i>atpD</i>
	324	<i>Listeria monocytogenes</i>	This patent	<i>atpD</i>
	325	<i>Micrococcus lylae</i>	This patent	<i>atpD</i>
25	326	<i>Moellerella wisconsensis</i>	This patent	<i>atpD</i>
	327	<i>Moraxella catarrhalis</i>	This patent	<i>atpD</i>
	328	<i>Moraxella osloensis</i>	This patent	<i>atpD</i>
	329	<i>Morganella morganii</i> subsp. <i>morganii</i>	This patent	<i>atpD</i>
	330	<i>Pantoea agglomerans</i>	This patent	<i>atpD</i>
30	331	<i>Pantoea dispersa</i>	This patent	<i>atpD</i>
	332	<i>Pasteurella multocida</i>	This patent	<i>atpD</i>
	333	<i>Pragia fontium</i>	This patent	<i>atpD</i>
	334	<i>Proteus mirabilis</i>	This patent	<i>atpD</i>
	335	<i>Proteus vulgaris</i>	This patent	<i>atpD</i>
35	336	<i>Providencia alcalifaciens</i>	This patent	<i>atpD</i>
	337	<i>Providencia rettgeri</i>	This patent	<i>atpD</i>
	338	<i>Providencia rustigianii</i>	This patent	<i>atpD</i>
	339	<i>Providencia stuartii</i>	This patent	<i>atpD</i>
	340	<i>Psychrobacter phenylpyruvicum</i>	This patent	<i>atpD</i>
40	341	<i>Rahnella aquatilis</i>	This patent	<i>atpD</i>
	342	<i>Salmonella choleraesuis</i> subsp. <i>arizonae</i>	This patent	<i>atpD</i>
	343	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Choleraesuis	This patent	<i>atpD</i>
	344	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	This patent	<i>atpD</i>
	345	<i>Salmonella choleraesuis</i> subsp. <i>houtenae</i>	This patent	<i>atpD</i>
45	346	<i>Salmonella choleraesuis</i> subsp. <i>indica</i>	This patent	<i>atpD</i>
	347	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Paratyphi A	This patent	<i>atpD</i>
	348	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Paratyphi B	This patent	<i>atpD</i>
50	349	<i>Salmonella choleraesuis</i> subsp. <i>salamae</i>	This patent	<i>atpD</i>
	350	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	This patent	<i>atpD</i>
	351	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	This patent	<i>atpD</i>
55	352	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Virchow	This patent	<i>atpD</i>
	353	<i>Serratia ficaria</i>	This patent	<i>atpD</i>
	354	<i>Serratia fonticola</i>	This patent	<i>atpD</i>
	355	<i>Serratia grimesii</i>	This patent	<i>atpD</i>
	356	<i>Serratia liquefaciens</i>	This patent	<i>atpD</i>
60	357	<i>Serratia marcescens</i>	This patent	<i>atpD</i>
	358	<i>Serratia odorifera</i>	This patent	<i>atpD</i>
	359	<i>Serratia plymuthica</i>	This patent	<i>atpD</i>
	360	<i>Serratia rubidaea</i>	This patent	<i>atpD</i>
	361	<i>Pseudomonas putida</i>	This patent	<i>atpD</i>
65	362	<i>Shigella boydii</i>	This patent	<i>atpD</i>
	363	<i>Shigella dysenteriae</i>	This patent	<i>atpD</i>

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	SourceGene*
5	364 <i>Shigella flexneri</i>	This patent <i>atpD</i>
	365 <i>Shigella sonnei</i>	This patent <i>atpD</i>
	366 <i>Staphylococcus aureus</i>	This patent <i>atpD</i>
	367 <i>Staphylococcus auricularis</i>	This patent <i>atpD</i>
	368 <i>Staphylococcus capitis</i> subsp. <i>capitis</i>	This patent <i>atpD</i>
10	369 <i>Staphylococcus cohnii</i> subsp. <i>cohnii</i>	This patent <i>atpD</i>
	370 <i>Staphylococcus epidermidis</i>	This patent <i>atpD</i>
	371 <i>Staphylococcus haemolyticus</i>	This patent <i>atpD</i>
	372 <i>Staphylococcus hominis</i> subsp. <i>hominis</i>	This patent <i>atpD</i>
	373 <i>Staphylococcus hominis</i>	This patent <i>atpD</i>
15	374 <i>Staphylococcus lugdunensis</i>	This patent <i>atpD</i>
	375 <i>Staphylococcus saprophyticus</i>	This patent <i>atpD</i>
	376 <i>Staphylococcus simulans</i>	This patent <i>atpD</i>
	377 <i>Staphylococcus warneri</i>	This patent <i>atpD</i>
	378 <i>Streptococcus acidominimus</i>	This patent <i>atpD</i>
20	379 <i>Streptococcus agalactiae</i>	This patent <i>atpD</i>
	380 <i>Streptococcus agalactiae</i>	This patent <i>atpD</i>
	381 <i>Streptococcus agalactiae</i>	This patent <i>atpD</i>
	382 <i>Streptococcus agalactiae</i>	This patent <i>atpD</i>
	383 <i>Streptococcus agalactiae</i>	This patent <i>atpD</i>
25	384 <i>Streptococcus dysgalactiae</i>	This patent <i>atpD</i>
	385 <i>Streptococcus equi</i> subsp. <i>equi</i>	This patent <i>atpD</i>
	386 <i>Streptococcus anginosus</i>	This patent <i>atpD</i>
	387 <i>Streptococcus salivarius</i>	This patent <i>atpD</i>
	388 <i>Streptococcus suis</i>	This patent <i>atpD</i>
30	389 <i>Streptococcus uberis</i>	This patent <i>atpD</i>
	390 <i>Tatumella ptyseos</i>	This patent <i>atpD</i>
	391 <i>Trabulsiella guamensis</i>	This patent <i>atpD</i>
	392 <i>Yersinia bercovieri</i>	This patent <i>atpD</i>
	393 <i>Yersinia enterocolitica</i>	This patent <i>atpD</i>
35	394 <i>Yersinia frederiksenii</i>	This patent <i>atpD</i>
	395 <i>Yersinia intermedia</i>	This patent <i>atpD</i>
	396 <i>Yersinia pseudotuberculosis</i>	This patent <i>atpD</i>
	397 <i>Yersinia rohdei</i>	This patent <i>atpD</i>
	398 <i>Yokenella regensburgei</i>	This patent <i>atpD</i>
40	399 <i>Yarrowia lipolytica</i>	This patent <i>tuf</i> (EF-1)
	400 <i>Absidia corymbifera</i>	This patent <i>tuf</i> (EF-1)
	401 <i>Alternaria alternata</i>	This patent <i>tuf</i> (EF-1)
	402 <i>Aspergillus flavus</i>	This patent <i>tuf</i> (EF-1)
	403 <i>Aspergillus fumigatus</i>	This patent <i>tuf</i> (EF-1)
45	404 <i>Aspergillus fumigatus</i>	This patent <i>tuf</i> (EF-1)
	405 <i>Aspergillus niger</i>	This patent <i>tuf</i> (EF-1)
	406 <i>Blastoschizomyces capitatus</i>	This patent <i>tuf</i> (EF-1)
	407 <i>Candida albicans</i>	This patent <i>tuf</i> (EF-1)
	408 <i>Candida albicans</i>	This patent <i>tuf</i> (EF-1)
50	409 <i>Candida albicans</i>	This patent <i>tuf</i> (EF-1)
	410 <i>Candida albicans</i>	This patent <i>tuf</i> (EF-1)
	411 <i>Candida albicans</i>	This patent <i>tuf</i> (EF-1)
	412 <i>Candida dubliniensis</i>	This patent <i>tuf</i> (EF-1)
	413 <i>Candida catenulata</i>	This patent <i>tuf</i> (EF-1)
55	414 <i>Candida dubliniensis</i>	This patent <i>tuf</i> (EF-1)
	415 <i>Candida dubliniensis</i>	This patent <i>tuf</i> (EF-1)
	416 <i>Candida famata</i>	This patent <i>tuf</i> (EF-1)
	417 <i>Candida glabrata</i>	WO98/20157 <i>tuf</i> (EF-1)
	418 <i>Candida guilliermondii</i>	This patent <i>tuf</i> (EF-1)
60	419 <i>Candida haemulonii</i>	This patent <i>tuf</i> (EF-1)
	420 <i>Candida inconspicua</i>	This patent <i>tuf</i> (EF-1)
	421 <i>Candida kefyr</i>	This patent <i>tuf</i> (EF-1)
	422 <i>Candida krusei</i>	WO98/20157 <i>tuf</i> (EF-1)
	423 <i>Candida lambica</i>	This patent <i>tuf</i> (EF-1)
65	424 <i>Candida lusitanae</i>	This patent <i>tuf</i> (EF-1)
	425 <i>Candida norvegensis</i>	This patent <i>tuf</i> (EF-1)

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	426	<i>Candida parapsilosis</i>	WO98/20157	<i>tuf</i> (EF-1)
	427	<i>Candida rugosa</i>	This patent	<i>tuf</i> (EF-1)
	428	<i>Candida sphaerica</i>	This patent	<i>tuf</i> (EF-1)
	429	<i>Candida tropicalis</i>	WO98/20157	<i>tuf</i> (EF-1)
	430	<i>Candida utilis</i>	This patent	<i>tuf</i> (EF-1)
10	431	<i>Candida viswanathii</i>	This patent	<i>tuf</i> (EF-1)
	432	<i>Candida zeylanoides</i>	This patent	<i>tuf</i> (EF-1)
	433	<i>Coccidioides immitis</i>	This patent	<i>tuf</i> (EF-1)
	434	<i>Cryptococcus albidus</i>	This patent	<i>tuf</i> (EF-1)
	435	<i>Exophiala jeanselmei</i>	This patent	<i>tuf</i> (EF-1)
15	436	<i>Fusarium oxysporum</i>	This patent	<i>tuf</i> (EF-1)
	437	<i>Geotrichum</i> sp.	This patent	<i>tuf</i> (EF-1)
	438	<i>Histoplasma capsulatum</i>	This patent	<i>tuf</i> (EF-1)
	439	<i>Issatchenkia orientalis</i> Kudrjanzev	This patent	<i>tuf</i> (EF-1)
	440	<i>Malassezia furfur</i>	This patent	<i>tuf</i> (EF-1)
20	441	<i>Malassezia pachydermatis</i>	This patent	<i>tuf</i> (EF-1)
	442	<i>Malbranchea filamentosa</i>	This patent	<i>tuf</i> (EF-1)
	443	<i>Metschnikowia pulcherrima</i>	This patent	<i>tuf</i> (EF-1)
	444	<i>Paecilomyces lilacinus</i>	This patent	<i>tuf</i> (EF-1)
	445	<i>Paracoccidioides brasiliensis</i>	This patent	<i>tuf</i> (EF-1)
25	446	<i>Penicillium marneffei</i>	This patent	<i>tuf</i> (EF-1)
	447	<i>Pichia anomala</i>	This patent	<i>tuf</i> (EF-1)
	448	<i>Pichia anomala</i>	This patent	<i>tuf</i> (EF-1)
	449	<i>Pseudallescheria boydii</i>	This patent	<i>tuf</i> (EF-1)
	450	<i>Rhizopus oryzae</i>	This patent	<i>tuf</i> (EF-1)
30	451	<i>Rhodotorula minuta</i>	This patent	<i>tuf</i> (EF-1)
	452	<i>Sporobolomyces salmonicolor</i>	This patent	<i>tuf</i> (EF-1)
	453	<i>Sporothrix schenckii</i>	This patent	<i>tuf</i> (EF-1)
	454	<i>Stephanoascus ciferrii</i>	This patent	<i>tuf</i> (EF-1)
	455	<i>Trichophyton mentagrophytes</i>	This patent	<i>tuf</i> (EF-1)
35	456	<i>Trichosporon cutaneum</i>	This patent	<i>tuf</i> (EF-1)
	457	<i>Wangiella dermatitidis</i>	This patent	<i>tuf</i> (EF-1)
	458	<i>Aspergillus fumigatus</i>	This patent	<i>atpD</i>
	459	<i>Blastoschizomyces capitatus</i>	This patent	<i>atpD</i>
	460	<i>Candida albicans</i>	This patent	<i>atpD</i>
40	461	<i>Candida dubliniensis</i>	This patent	<i>atpD</i>
	462	<i>Candida famata</i>	This patent	<i>atpD</i>
	463	<i>Candida glabrata</i>	This patent	<i>atpD</i>
	464	<i>Candida guilliermondii</i>	This patent	<i>atpD</i>
	465	<i>Candida haemulonii</i>	This patent	<i>atpD</i>
45	466	<i>Candida inconspicua</i>	This patent	<i>atpD</i>
	467	<i>Candida kefyr</i>	This patent	<i>atpD</i>
	468	<i>Candida krusei</i>	This patent	<i>atpD</i>
	469	<i>Candida lambica</i>	This patent	<i>atpD</i>
	470	<i>Candida lusitanae</i>	This patent	<i>atpD</i>
50	471	<i>Candida norvegensis</i>	This patent	<i>atpD</i>
	472	<i>Candida parapsilosis</i>	This patent	<i>atpD</i>
	473	<i>Candida rugosa</i>	This patent	<i>atpD</i>
	474	<i>Candida sphaerica</i>	This patent	<i>atpD</i>
	475	<i>Candida tropicalis</i>	This patent	<i>atpD</i>
55	476	<i>Candida utilis</i>	This patent	<i>atpD</i>
	477	<i>Candida viswanathii</i>	This patent	<i>atpD</i>
	478	<i>Candida zeylanoides</i>	This patent	<i>atpD</i>
	479	<i>Coccidioides immitis</i>	This patent	<i>atpD</i>
	480	<i>Cryptococcus albidus</i>	This patent	<i>atpD</i>
60	481	<i>Fusarium oxysporum</i>	This patent	<i>atpD</i>
	482	<i>Geotrichum</i> sp.	This patent	<i>atpD</i>
	483	<i>Histoplasma capsulatum</i>	This patent	<i>atpD</i>
	484	<i>Malassezia furfur</i>	This patent	<i>atpD</i>
	485	<i>Malassezia pachydermatis</i>	This patent	<i>atpD</i>
65	486	<i>Metschnikowia pulcherrima</i>	This patent	<i>atpD</i>
	487	<i>Penicillium marneffei</i>	This patent	<i>atpD</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	488 <i>Pichia anomala</i>	This patent	<i>atpD</i>
	489 <i>Pichia anomala</i>	This patent	<i>atpD</i>
	490 <i>Rhodotorula minuta</i>	This patent	<i>atpD</i>
	491 <i>Rhodotorula mucilaginosa</i>	This patent	<i>atpD</i>
	492 <i>Sporobolomyces salmonicolor</i>	This patent	<i>atpD</i>
10	493 <i>Sporothrix schenckii</i>	This patent	<i>atpD</i>
	494 <i>Stephanoascus ciferrii</i>	This patent	<i>atpD</i>
	495 <i>Trichophyton mentagrophytes</i>	This patent	<i>atpD</i>
	496 <i>Wangiella dermatitidis</i>	This patent	<i>atpD</i>
	497 <i>Yarrowia lipolytica</i>	This patent	<i>atpD</i>
15	498 <i>Aspergillus fumigatus</i>	This patent	<i>tuf</i> (M)
	499 <i>Blastoschizomyces capitatus</i>	This patent	<i>tuf</i> (M)
	500 <i>Candida rugosa</i>	This patent	<i>tuf</i> (M)
	501 <i>Coccidioides immitis</i>	This patent	<i>tuf</i> (M)
	502 <i>Fusarium oxysporum</i>	This patent	<i>tuf</i> (M)
20	503 <i>Histoplasma capsulatum</i>	This patent	<i>tuf</i> (M)
	504 <i>Paracoccidioides brasiliensis</i>	This patent	<i>tuf</i> (M)
	505 <i>Penicillium marneffei</i>	This patent	<i>tuf</i> (M)
	506 <i>Pichia anomala</i>	This patent	<i>tuf</i> (M)
	507 <i>Trichophyton mentagrophytes</i>	This patent	<i>tuf</i> (M)
25	508 <i>Yarrowia lipolytica</i>	This patent	<i>tuf</i> (M)
	509 <i>Babesia bigemina</i>	This patent	<i>tuf</i> (EF-1)
	510 <i>Babesia bovis</i>	This patent	<i>tuf</i> (EF-1)
	511 <i>Crithidia fasciculata</i>	This patent	<i>tuf</i> (EF-1)
	512 <i>Entamoeba histolytica</i>	This patent	<i>tuf</i> (EF-1)
30	513 <i>Giardia lamblia</i>	This patent	<i>tuf</i> (EF-1)
	514 <i>Leishmania tropica</i>	This patent	<i>tuf</i> (EF-1)
	515 <i>Leishmania aethiopica</i>	This patent	<i>tuf</i> (EF-1)
	516 <i>Leishmania tropica</i>	This patent	<i>tuf</i> (EF-1)
	517 <i>Leishmania donovani</i>	This patent	<i>tuf</i> (EF-1)
35	518 <i>Leishmania infantum</i>	This patent	<i>tuf</i> (EF-1)
	519 <i>Leishmania enriettii</i>	This patent	<i>tuf</i> (EF-1)
	520 <i>Leishmania gerbilli</i>	This patent	<i>tuf</i> (EF-1)
	521 <i>Leishmania hertigi</i>	This patent	<i>tuf</i> (EF-1)
	522 <i>Leishmania major</i>	This patent	<i>tuf</i> (EF-1)
40	523 <i>Leishmania amazonensis</i>	This patent	<i>tuf</i> (EF-1)
	524 <i>Leishmania mexicana</i>	This patent	<i>tuf</i> (EF-1)
	525 <i>Leishmania tarentolae</i>	This patent	<i>tuf</i> (EF-1)
	526 <i>Leishmania tropica</i>	This patent	<i>tuf</i> (EF-1)
	527 <i>Neospora caninum</i>	This patent	<i>tuf</i> (EF-1)
45	528 <i>Trichomonas vaginalis</i>	This patent	<i>tuf</i> (EF-1)
	529 <i>Trypanosoma brucei</i> subsp. <i>brucei</i>	This patent	<i>tuf</i> (EF-1)
	530 <i>Crithidia fasciculata</i>	This patent	<i>atpD</i>
	531 <i>Leishmania tropica</i>	This patent	<i>atpD</i>
	532 <i>Leishmania aethiopica</i>	This patent	<i>atpD</i>
50	533 <i>Leishmania donovani</i>	This patent	<i>atpD</i>
	534 <i>Leishmania infantum</i>	This patent	<i>atpD</i>
	535 <i>Leishmania gerbilli</i>	This patent	<i>atpD</i>
	536 <i>Leishmania hertigi</i>	This patent	<i>atpD</i>
	537 <i>Leishmania major</i>	This patent	<i>atpD</i>
55	538 <i>Leishmania amazonensis</i>	This patent	<i>atpD</i>
	607 <i>Enterococcus faecalis</i>	WO98/20157	<i>tuf</i>
	608 <i>Enterococcus faecium</i>	WO98/20157	<i>tuf</i>
	609 <i>Enterococcus gallinarum</i>	WO98/20157	<i>tuf</i>
	610 <i>Haemophilus influenzae</i>	WO98/20157	<i>tuf</i>
60	611 <i>Staphylococcus epidermidis</i>	WO98/20157	<i>tuf</i>
	612 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i>	This patent	<i>tuf</i>
	serotype Paratyphi A		
	613 <i>Serratia ficaria</i>	This patent	<i>tuf</i>
	614 <i>Enterococcus malodoratus</i>	This patent	<i>tuf</i> (C)
65	615 <i>Enterococcus durans</i>	This patent	<i>tuf</i> (C)
	616 <i>Enterococcus pseudoavium</i>	This patent	<i>tuf</i> (C)

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	617	<i>Enterococcus dispar</i>	This patent	<i>tuf</i> (C)
	618	<i>Enterococcus avium</i>	This patent	<i>tuf</i> (C)
	619	<i>Saccharomyces cerevisiae</i>	Database	<i>tuf</i> (M)
	621	<i>Enterococcus faecium</i>	This patent	<i>tuf</i> (C)
	622	<i>Saccharomyces cerevisiae</i>	This patent	<i>tuf</i> (EF-1)
10	623	<i>Cryptococcus neoformans</i>	This patent	<i>tuf</i> (EF-1)
	624	<i>Candida albicans</i>	WO98/20157	<i>tuf</i> (EF-1)
	662	<i>Corynebacterium diphtheriae</i>	WO98/20157	<i>tuf</i>
	663	<i>Candida catenulata</i>	This patent	<i>atpD</i>
	665	<i>Saccharomyces cerevisiae</i>	Database	<i>tuf</i> (EF-1)
15	666	<i>Saccharomyces cerevisiae</i>	Database	<i>atpD</i>
	667	<i>Trypanosoma cruzi</i>	This patent	<i>atpD</i>
	668	<i>Corynebacterium glutamicum</i>	Database	<i>tuf</i>
	669	<i>Escherichia coli</i>	Database	<i>atpD</i>
	670	<i>Helicobacter pylori</i>	Database	<i>atpD</i>
20	671	<i>Clostridium acetobutylicum</i>	Database	<i>atpD</i>
	672	<i>Cytophaga lytica</i>	Database	<i>atpD</i>
	673	<i>Ehrlichia risticii</i>	This patent	<i>atpD</i>
	674	<i>Vibrio cholerae</i>	This patent	<i>atpD</i>
	675	<i>Vibrio cholerae</i>	This patent	<i>tuf</i>
25	676	<i>Leishmania enriettii</i>	This patent	<i>atpD</i>
	677	<i>Babesia microti</i>	This patent	<i>tuf</i> (EF-1)
	678	<i>Cryptococcus neoformans</i>	This patent	<i>atpD</i>
	679	<i>Cryptococcus neoformans</i>	This patent	<i>atpD</i>
	680	<i>Cunninghamella bertholletiae</i>	This patent	<i>atpD</i>
30	684	<i>Candida tropicalis</i>	Database	<i>atpD</i> (V)
	685	<i>Enterococcus hirae</i>	Database	<i>atpD</i> (V)
	686	<i>Chlamydia pneumoniae</i>	Database	<i>atpD</i> (V)
	687	<i>Halobacterium salinarum</i>	Database	<i>atpD</i> (V)
	688	<i>Homo sapiens</i>	Database	<i>atpD</i> (V)
35	689	<i>Plasmodium falciparum</i>	Database	<i>atpD</i> (V)
	690	<i>Saccharomyces cerevisiae</i>	Database	<i>atpD</i> (V)
	691	<i>Schizosaccharomyces pombe</i>	Database	<i>atpD</i> (V)
	692	<i>Trypanosoma congolense</i>	Database	<i>atpD</i> (V)
	693	<i>Thermus thermophilus</i>	Database	<i>atpD</i> (V)
40	698	<i>Escherichia coli</i>	WO98/20157	<i>tuf</i>
	709	<i>Borrelia burgdorferi</i>	Database	<i>atpD</i> (V)
	710	<i>Treponema pallidum</i>	Database	<i>atpD</i> (V)
	711	<i>Chlamydia trachomatis</i>	Genome project	<i>atpD</i> (V)
	712	<i>Enterococcus faecalis</i>	Genome project	<i>atpD</i> (V)
45	713	<i>Methanosarcina barkeri</i>	Database	<i>atpD</i> (V)
	714	<i>Methanococcus jannaschii</i>	Database	<i>atpD</i> (V)
	715	<i>Porphyromonas gingivalis</i>	Genome project	<i>atpD</i> (V)
	716	<i>Streptococcus pneumoniae</i>	Genome project	<i>atpD</i> (V)
	717	<i>Burkholderia mallei</i>	This patent	<i>tuf</i>
50	718	<i>Burkholderia pseudomallei</i>	This patent	<i>tuf</i>
	719	<i>Clostridium beijerinckii</i>	This patent	<i>tuf</i>
	720	<i>Clostridium innocuum</i>	This patent	<i>tuf</i>
	721	<i>Clostridium novyi</i>	This patent	<i>tuf</i>
	722	<i>Clostridium septicum</i>	This patent	<i>tuf</i>
55	723	<i>Clostridium tertium</i>	This patent	<i>tuf</i>
	724	<i>Clostridium tetani</i>	This patent	<i>tuf</i>
	725	<i>Enterococcus malodoratus</i>	This patent	<i>tuf</i>
	726	<i>Enterococcus sulfureus</i>	This patent	<i>tuf</i>
	727	<i>Lactococcus garvieae</i>	This patent	<i>tuf</i>
60	728	<i>Mycoplasma pirum</i>	This patent	<i>tuf</i>
	729	<i>Mycoplasma salivarium</i>	This patent	<i>tuf</i>
	730	<i>Neisseria polysaccharea</i>	This patent	<i>tuf</i>
	731	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i>	This patent	<i>tuf</i>
		serotype Enteritidis		

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	732 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Gallinarum	This patent	<i>tuf</i>
	733 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Paratyphi B	This patent	<i>tuf</i>
10	734 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Virchow	This patent	<i>tuf</i>
	735 <i>Serratia grimesii</i>	This patent	<i>tuf</i>
	736 <i>Clostridium difficile</i>	This patent	<i>tuf</i>
	737 <i>Burkholderia pseudomallei</i>	This patent	<i>atpD</i>
	738 <i>Clostridium bifermentans</i>	This patent	<i>atpD</i>
15	739 <i>Clostridium beijerinckii</i>	This patent	<i>atpD</i>
	740 <i>Clostridium difficile</i>	This patent	<i>atpD</i>
	741 <i>Clostridium ramosum</i>	This patent	<i>atpD</i>
	742 <i>Clostridium septicum</i>	This patent	<i>atpD</i>
	743 <i>Clostridium tertium</i>	This patent	<i>atpD</i>
20	744 <i>Comamonas acidovorans</i>	This patent	<i>atpD</i>
	745 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>	This patent	<i>atpD</i>
	746 <i>Neisseria canis</i>	This patent	<i>atpD</i>
	747 <i>Neisseria cinerea</i>	This patent	<i>atpD</i>
	748 <i>Neisseria cuniculi</i>	This patent	<i>atpD</i>
25	749 <i>Neisseria elongata</i> subsp. <i>elongata</i>	This patent	<i>atpD</i>
	750 <i>Neisseria flavescens</i>	This patent	<i>atpD</i>
	751 <i>Neisseria gonorrhoeae</i>	This patent	<i>atpD</i>
	752 <i>Neisseria gonorrhoeae</i>	This patent	<i>atpD</i>
	753 <i>Neisseria lactamica</i>	This patent	<i>atpD</i>
30	754 <i>Neisseria meningitidis</i>	This patent	<i>atpD</i>
	755 <i>Neisseria mucosa</i>	This patent	<i>atpD</i>
	756 <i>Neisseria subflava</i>	This patent	<i>atpD</i>
	757 <i>Neisseria weaveri</i>	This patent	<i>atpD</i>
	758 <i>Neisseria animalis</i>	This patent	<i>atpD</i>
35	759 <i>Proteus penneri</i>	This patent	<i>atpD</i>
	760 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Enteritidis	This patent	<i>atpD</i>
	761 <i>Yersinia pestis</i>	This patent	<i>atpD</i>
40	762 <i>Burkholderia mallei</i>	This patent	<i>atpD</i>
	763 <i>Clostridium sordellii</i>	This patent	<i>atpD</i>
	764 <i>Clostridium novyi</i>	This patent	<i>atpD</i>
	765 <i>Clostridium botulinum</i>	This patent	<i>atpD</i>
	766 <i>Clostridium histolyticum</i>	This patent	<i>atpD</i>
	767 <i>Peptostreptococcus prevotii</i>	This patent	<i>atpD</i>
45	768 <i>Absidia corymbifera</i>	This patent	<i>atpD</i>
	769 <i>Alternaria alternata</i>	This patent	<i>atpD</i>
	770 <i>Aspergillus flavus</i>	This patent	<i>atpD</i>
	771 <i>Mucor circinelloides</i>	This patent	<i>atpD</i>
	772 <i>Piedraia hortai</i>	This patent	<i>atpD</i>
50	773 <i>Pseudallescheria boydii</i>	This patent	<i>atpD</i>
	774 <i>Rhizopus oryzae</i>	This patent	<i>atpD</i>
	775 <i>Scopulariopsis koningii</i>	This patent	<i>atpD</i>
	776 <i>Trichophyton mentagrophytes</i>	This patent	<i>atpD</i>
	777 <i>Trichophyton tonsurans</i>	This patent	<i>atpD</i>
55	778 <i>Trichosporon cutaneum</i>	This patent	<i>atpD</i>
	779 <i>Cladophialophora carrionii</i>	This patent	<i>tuf</i> (EF-1)
	780 <i>Cunninghamella bertholletiae</i>	This patent	<i>tuf</i> (EF-1)
	781 <i>Curvularia lunata</i>	This patent	<i>tuf</i> (EF-1)
	782 <i>Fonsecaea pedrosoi</i>	This patent	<i>tuf</i> (EF-1)
60	783 <i>Microsporum audouinii</i>	This patent	<i>tuf</i> (EF-1)
	784 <i>Mucor circinelloides</i>	This patent	<i>tuf</i> (EF-1)
	785 <i>Phialophora verrucosa</i>	This patent	<i>tuf</i> (EF-1)
	786 <i>Saksenaea vasiformis</i>	This patent	<i>tuf</i> (EF-1)
	787 <i>Syncephalastrum racemosum</i>	This patent	<i>tuf</i> (EF-1)
65	788 <i>Trichophyton tonsurans</i>	This patent	<i>tuf</i> (EF-1)
	789 <i>Trichophyton mentagrophytes</i>	This patent	<i>tuf</i> (EF-1)

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	790 <i>Bipolaris hawaiiensis</i>	This patent	<i>tuf</i> (EF-1)
	791 <i>Aspergillus fumigatus</i>	This patent	<i>tuf</i> (M)
	792 <i>Trichophyton mentagrophytes</i>	This patent	<i>tuf</i> (M)
	827 <i>Clostridium novyi</i>	This patent	<i>atpD</i> (V)
10	828 <i>Clostridium difficile</i>	This patent	<i>atpD</i> (V)
	829 <i>Clostridium septicum</i>	This patent	<i>atpD</i> (V)
	830 <i>Clostridium botulinum</i>	This patent	<i>atpD</i> (V)
	831 <i>Clostridium perfringens</i>	This patent	<i>atpD</i> (V)
15	832 <i>Clostridium tetani</i>	This patent	<i>atpD</i> (V)
	833 <i>Streptococcus pyogenes</i>	Database	<i>atpD</i> (V)
	834 <i>Babesia bovis</i>	This patent	<i>atpD</i> (V)
	835 <i>Cryptosporidium parvum</i>	This patent	<i>atpD</i> (V)
20	836 <i>Leishmania infantum</i>	This patent	<i>atpD</i> (V)
	837 <i>Leishmania major</i>	This patent	<i>atpD</i> (V)
	838 <i>Leishmania tarentolae</i>	This patent	<i>atpD</i> (V)
	839 <i>Trypanosoma brucei</i>	This patent	<i>atpD</i> (V)
25	840 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (EF-1)
	841 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (EF-1)
	842 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (EF-1)
	843 <i>Babesia bovis</i>	This patent	<i>tuf</i> (M)
30	844 <i>Leishmania aethiopica</i>	This patent	<i>tuf</i> (M)
	845 <i>Leishmania amazonensis</i>	This patent	<i>tuf</i> (M)
	846 <i>Leishmania donovani</i>	This patent	<i>tuf</i> (M)
	847 <i>Leishmania infantum</i>	This patent	<i>tuf</i> (M)
35	848 <i>Leishmania enriettii</i>	This patent	<i>tuf</i> (M)
	849 <i>Leishmania gerbilli</i>	This patent	<i>tuf</i> (M)
	850 <i>Leishmania major</i>	This patent	<i>tuf</i> (M)
	851 <i>Leishmania mexicana</i>	This patent	<i>tuf</i> (M)
40	852 <i>Leishmania tarentolae</i>	This patent	<i>tuf</i> (M)
	853 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (M)
	854 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (M)
	855 <i>Trypanosoma cruzi</i>	This patent	<i>tuf</i> (M)
45	856 <i>Babesia bigemina</i>	This patent	<i>atpD</i>
	857 <i>Babesia bovis</i>	This patent	<i>atpD</i>
	858 <i>Babesia microti</i>	This patent	<i>atpD</i>
	859 <i>Leishmania guyanensis</i>	This patent	<i>atpD</i>
50	860 <i>Leishmania mexicana</i>	This patent	<i>atpD</i>
	861 <i>Leishmania tropica</i>	This patent	<i>atpD</i>
	862 <i>Leishmania tropica</i>	This patent	<i>atpD</i>
	863 <i>Bordetella pertussis</i>	Database	<i>tuf</i>
55	864 <i>Trypanosoma brucei brucei</i>	Database	<i>tuf</i> (EF-1)
	865 <i>Cryptosporidium parvum</i>	This patent	<i>tuf</i> (EF-1)
	866 <i>Staphylococcus saprophyticus</i>	This patent	<i>atpD</i>
	867 <i>Zoogloea ramigera</i>	This patent	<i>atpD</i>
60	868 <i>Staphylococcus saprophyticus</i>	This patent	<i>tuf</i>
	869 <i>Enterococcus casseliflavus</i>	This patent	<i>tuf</i>
	870 <i>Enterococcus casseliflavus</i>	This patent	<i>tuf</i>
	871 <i>Enterococcus flavescens</i>	This patent	<i>tuf</i>
65	872 <i>Enterococcus gallinarum</i>	This patent	<i>tuf</i>
	873 <i>Enterococcus gallinarum</i>	This patent	<i>tuf</i>
	874 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	875 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
65	876 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
	877 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
	878 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
	879 <i>Enterococcus gallinarum</i>	This patent	<i>tuf</i>
65	880 <i>Pseudomonas aeruginosa</i>	This patent	<i>tuf</i>
	881 <i>Enterococcus casseliflavus</i>	This patent	<i>tuf</i>
	882 <i>Enterococcus casseliflavus</i>	This patent	<i>tuf</i>
	883 <i>Enterococcus faecalis</i>	This patent	<i>tuf</i>
65	884 <i>Enterococcus faecalis</i>	This patent	<i>tuf</i>
	885 <i>Enterococcus faecium</i>	This patent	<i>tuf</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	886	<i>Enterococcus faecium</i>	This patent	<i>tuf</i>
	887	<i>Zoogloea ramigera</i>	This patent	<i>tuf</i>
	888	<i>Enterococcus faecalis</i>	This patent	<i>tuf</i>
	889	<i>Aspergillus fumigatus</i>	This patent	<i>atpD</i>
	890	<i>Penicillium marneffei</i>	This patent	<i>atpD</i>
10	891	<i>Paecilomyces lilacinus</i>	This patent	<i>atpD</i>
	892	<i>Penicillium marneffei</i>	This patent	<i>atpD</i>
	893	<i>Sporothrix schenckii</i>	This patent	<i>atpD</i>
	894	<i>Malbranchea filamentosa</i>	This patent	<i>atpD</i>
	895	<i>Paecilomyces lilacinus</i>	This patent	<i>atpD</i>
15	896	<i>Aspergillus niger</i>	This patent	<i>atpD</i>
	897	<i>Aspergillus fumigatus</i>	This patent	<i>tuf</i> (EF-1)
	898	<i>Penicillium marneffei</i>	This patent	<i>tuf</i> (EF-1)
	899	<i>Piedraia hortai</i>	This patent	<i>tuf</i> (EF-1)
	900	<i>Paecilomyces lilacinus</i>	This patent	<i>tuf</i> (EF-1)
20	901	<i>Paracoccidioides brasiliensis</i>	This patent	<i>tuf</i> (EF-1)
	902	<i>Sporothrix schenckii</i>	This patent	<i>tuf</i> (EF-1)
	903	<i>Penicillium marneffei</i>	This patent	<i>tuf</i> (EF-1)
	904	<i>Curvularia lunata</i>	This patent	<i>tuf</i> (M)
	905	<i>Aspergillus niger</i>	This patent	<i>tuf</i> (M)
25	906	<i>Bipolaris hawaiiensis</i>	This patent	<i>tuf</i> (M)
	907	<i>Aspergillus flavus</i>	This patent	<i>tuf</i> (M)
	908	<i>Alternaria alternata</i>	This patent	<i>tuf</i> (M)
	909	<i>Penicillium marneffei</i>	This patent	<i>tuf</i> (M)
	910	<i>Penicillium marneffei</i>	This patent	<i>tuf</i> (M)
30	918	<i>Escherichia coli</i>	Database	<i>recA</i>
	929	<i>Bacteroides fragilis</i>	This patent	<i>atpD</i> (V)
	930	<i>Bacteroides distasonis</i>	This patent	<i>atpD</i> (V)
	931	<i>Porphyromonas asaccharolytica</i>	This patent	<i>atpD</i> (V)
	932	<i>Listeria monocytogenes</i>	This patent	<i>tuf</i>
35	939	<i>Saccharomyces cerevisiae</i>	Database	<i>recA</i> (Rad51)
	940	<i>Saccharomyces cerevisiae</i>	Database	<i>recA</i> (Dmc1)
	941	<i>Cryptococcus humicola</i>	This patent	<i>atpD</i>
	942	<i>Escherichia coli</i>	This patent	<i>atpD</i>
	943	<i>Escherichia coli</i>	This patent	<i>atpD</i>
40	944	<i>Escherichia coli</i>	This patent	<i>atpD</i>
	945	<i>Escherichia coli</i>	This patent	<i>atpD</i>
	946	<i>Neisseria polysaccharea</i>	This patent	<i>atpD</i>
	947	<i>Neisseria sicca</i>	This patent	<i>atpD</i>
	948	<i>Streptococcus mitis</i>	This patent	<i>atpD</i>
45	949	<i>Streptococcus mitis</i>	This patent	<i>atpD</i>
	950	<i>Streptococcus mitis</i>	This patent	<i>atpD</i>
	951	<i>Streptococcus oralis</i>	This patent	<i>atpD</i>
	952	<i>Streptococcus pneumoniae</i>	This patent	<i>atpD</i>
	953	<i>Streptococcus pneumoniae</i>	This patent	<i>atpD</i>
50	954	<i>Streptococcus pneumoniae</i>	This patent	<i>atpD</i>
	955	<i>Streptococcus pneumoniae</i>	This patent	<i>atpD</i>
	956	<i>Babesia microti</i>	This patent	<i>atpD</i> (V)
	957	<i>Entamoeba histolytica</i>	This patent	<i>atpD</i> (V)
	958	<i>Fusobacterium nucleatum</i> subsp. <i>polymorphum</i>	This patent	<i>atpD</i> (V)
55	959	<i>Leishmania aethiopica</i>	This patent	<i>atpD</i> (V)
	960	<i>Leishmania tropica</i>	This patent	<i>atpD</i> (V)
	961	<i>Leishmania guyanensis</i>	This patent	<i>atpD</i> (V)
	962	<i>Leishmania donovani</i>	This patent	<i>atpD</i> (V)
	963	<i>Leishmania hertigi</i>	This patent	<i>atpD</i> (V)
60	964	<i>Leishmania mexicana</i>	This patent	<i>atpD</i> (V)
	965	<i>Leishmania tropica</i>	This patent	<i>atpD</i> (V)
	966	<i>Peptostreptococcus anaerobius</i>	This patent	<i>atpD</i> (V)
	967	<i>Bordetella pertussis</i>	This patent	<i>tuf</i>
	968	<i>Bordetella pertussis</i>	This patent	<i>tuf</i>
65	969	<i>Enterococcus columbae</i>	This patent	<i>tuf</i>

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	970	<i>Enterococcus flavescens</i>	This patent	<i>tuf</i>
	971	<i>Streptococcus pneumoniae</i>	This patent	<i>tuf</i>
	972	<i>Escherichia coli</i>	This patent	<i>tuf</i>
	973	<i>Escherichia coli</i>	This patent	<i>tuf</i>
	974	<i>Escherichia coli</i>	This patent	<i>tuf</i>
10	975	<i>Escherichia coli</i>	This patent	<i>tuf</i>
	976	<i>Mycobacterium avium</i>	This patent	<i>tuf</i>
	977	<i>Streptococcus pneumoniae</i>	This patent	<i>tuf</i>
	978	<i>Mycobacterium gordonae</i>	This patent	<i>tuf</i>
	979	<i>Streptococcus pneumoniae</i>	This patent	<i>tuf</i>
15	980	<i>Mycobacterium tuberculosis</i>	This patent	<i>tuf</i>
	981	<i>Staphylococcus warneri</i>	This patent	<i>tuf</i>
	982	<i>Streptococcus mitis</i>	This patent	<i>tuf</i>
	983	<i>Streptococcus mitis</i>	This patent	<i>tuf</i>
	984	<i>Streptococcus mitis</i>	This patent	<i>tuf</i>
20	985	<i>Streptococcus oralis</i>	This patent	<i>tuf</i>
	986	<i>Streptococcus pneumoniae</i>	This patent	<i>tuf</i>
	987	<i>Enterococcus hirae</i>	This patent	<i>tuf</i> (C)
	988	<i>Enterococcus mundtii</i>	This patent	<i>tuf</i> (C)
	989	<i>Enterococcus raffinosus</i>	This patent	<i>tuf</i> (C)
25	990	<i>Bacillus anthracis</i>	This patent	<i>recA</i>
	991	<i>Prevotella melaninogenica</i>	This patent	<i>recA</i>
	992	<i>Enterococcus casseliflavus</i>	This patent	<i>tuf</i>
	993	<i>Streptococcus pyogenes</i>	Database	<i>speA</i>
	1002	<i>Streptococcus pyogenes</i>	WO98/20157	<i>tuf</i>
30	1003	<i>Bacillus cereus</i>	This patent	<i>recA</i>
	1004	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1005	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1006	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1007	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
35	1008	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1009	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1010	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1011	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1012	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
40	1013	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1014	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1015	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1016	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1017	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
45	1018	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1019	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1020	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1021	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1022	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
50	1023	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1024	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1025	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1026	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1027	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
55	1028	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1029	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1030	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1031	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1032	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
60	1033	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2b</i>
	1034	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1035	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1036	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1037	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitological species	Source	Gene*
5	1038	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1039	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1040	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1041	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1042	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
10	1043	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1044	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1045	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1046	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1047	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
15	1048	<i>Streptococcus pneumoniae</i>	This patent	<i>pbp2x</i>
	1049	<i>Enterococcus faecium</i>	This patent	<i>vanA</i>
	1050	<i>Enterococcus gallinarum</i>	This patent	<i>vanA</i>
	1051	<i>Enterococcus faecium</i>	This patent	<i>vanA</i>
	1052	<i>Enterococcus faecium</i>	This patent	<i>vanA</i>
20	1053	<i>Enterococcus faecium</i>	This patent	<i>vanA</i>
	1054	<i>Enterococcus faecalis</i>	This patent	<i>vanA</i>
	1055	<i>Enterococcus gallinarum</i>	This patent	<i>vanA</i>
	1056	<i>Enterococcus faecium</i>	This patent	<i>vanA</i>
	1057	<i>Enterococcus flavescens</i>	This patent	<i>vanA</i>
25	1058	<i>Enterococcus gallinarum</i>	This patent	<i>vanC1</i>
	1059	<i>Enterococcus gallinarum</i>	This patent	<i>vanC1</i>
	1060	<i>Enterococcus casseliflavus</i>	This patent	<i>vanC2</i>
	1061	<i>Enterococcus casseliflavus</i>	This patent	<i>vanC2</i>
	1062	<i>Enterococcus casseliflavus</i>	This patent	<i>vanC2</i>
30	1063	<i>Enterococcus casseliflavus</i>	This patent	<i>vanC2</i>
	1064	<i>Enterococcus flavescens</i>	This patent	<i>vanC3</i>
	1065	<i>Enterococcus flavescens</i>	This patent	<i>vanC3</i>
	1066	<i>Enterococcus flavescens</i>	This patent	<i>vanC3</i>
	1067	<i>Enterococcus faecium</i>	This patent	<i>vanXY</i>
35	1068	<i>Enterococcus faecium</i>	This patent	<i>vanXY</i>
	1069	<i>Enterococcus faecium</i>	This patent	<i>vanXY</i>
	1070	<i>Enterococcus faecalis</i>	This patent	<i>vanXY</i>
	1071	<i>Enterococcus gallinarum</i>	This patent	<i>vanXY</i>
	1072	<i>Enterococcus faecium</i>	This patent	<i>vanXY</i>
40	1073	<i>Enterococcus flavescens</i>	This patent	<i>vanXY</i>
	1074	<i>Enterococcus faecium</i>	This patent	<i>vanXY</i>
	1075	<i>Enterococcus gallinarum</i>	This patent	<i>vanXY</i>
	1076	<i>Escherichia coli</i>	Database	<i>stx₁</i>
	1077	<i>Escherichia coli</i>	Database	<i>stx₂</i>
45	1093	<i>Staphylococcus saprophyticus</i>	This patent	unknown
	1117	<i>Enterococcus faecium</i>	Database	<i>vanB</i>
	1138	<i>Enterococcus gallinarum</i>	Database	<i>vanC1</i>
	1139	<i>Enterococcus faecium</i>	Database	<i>vanA</i>
	1140	<i>Enterococcus casseliflavus</i>	Database	<i>vanC2</i>
50	1141	<i>Enterococcus faecium</i>	Database	<i>vanHAXY</i>
	1169	<i>Streptococcus pneumoniae</i>	Database	<i>pbp1a</i>
	1172	<i>Streptococcus pneumoniae</i>	Database	<i>pbp2b</i>
	1173	<i>Streptococcus pneumoniae</i>	Database	<i>pbp2x</i>
	1178	<i>Staphylococcus aureus</i>	Database	<i>mecA</i>
55	1183	<i>Streptococcus pneumoniae</i>	Database	<i>hexA</i>
	1184	<i>Streptococcus pneumoniae</i>	This patent	<i>hexA</i>
	1185	<i>Streptococcus pneumoniae</i>	This patent	<i>hexA</i>
	1186	<i>Streptococcus pneumoniae</i>	This patent	<i>hexA</i>
	1187	<i>Streptococcus pneumoniae</i>	This patent	<i>hexA</i>
60				

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1188	<i>Streptococcus oralis</i>	This patent	<i>hexA</i>
	1189	<i>Streptococcus mitis</i>	This patent	<i>hexA</i>
	1190	<i>Streptococcus mitis</i>	This patent	<i>hexA</i>
	1191	<i>Streptococcus mitis</i>	This patent	<i>hexA</i>
	1198	<i>Staphylococcus saprophyticus</i>	This patent	unknown
10	1215	<i>Streptococcus pyogenes</i>	Database	<i>pcp</i>
	1230	<i>Escherichia coli</i>	Database	<i>tuf</i> (EF-G)
	1242	<i>Enterococcus faecium</i>	Database	<i>ddl</i>
	1243	<i>Enterococcus faecalis</i>	Database	<i>mtlF</i> , <i>mtlD</i>
	1244	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	This patent	unknown
15	1245	<i>Bacillus anthracis</i>	This patent	<i>atpD</i>
	1246	<i>Bacillus mycoides</i>	This patent	<i>atpD</i>
	1247	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1248	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1249	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
20	1250	<i>Bacillus weihenstephanensis</i>	This patent	<i>atpD</i>
	1251	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1252	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1253	<i>Bacillus cereus</i>	This patent	<i>atpD</i>
	1254	<i>Bacillus cereus</i>	This patent	<i>atpD</i>
25	1255	<i>Staphylococcus aureus</i>	This patent	<i>gyrA</i>
	1256	<i>Bacillus weihenstephanensis</i>	This patent	<i>atpD</i>
	1257	<i>Bacillus anthracis</i>	This patent	<i>atpD</i>
	1258	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1259	<i>Bacillus cereus</i>	This patent	<i>atpD</i>
30	1260	<i>Bacillus cereus</i>	This patent	<i>atpD</i>
	1261	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1262	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1263	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
	1264	<i>Bacillus thuringiensis</i>	This patent	<i>atpD</i>
35	1265	<i>Bacillus anthracis</i>	This patent	<i>atpD</i>
	1266	<i>Paracoccidioides brasiliensis</i>	This patent	<i>tuf</i> (EF-1)
	1267	<i>Blastomyces dermatitidis</i>	This patent	<i>tuf</i> (EF-1)
	1268	<i>Histoplasma capsulatum</i>	This patent	<i>tuf</i> (EF-1)
	1269	<i>Trichophyton rubrum</i>	This patent	<i>tuf</i> (EF-1)
40	1270	<i>Microsporum canis</i>	This patent	<i>tuf</i> (EF-1)
	1271	<i>Aspergillus versicolor</i>	This patent	<i>tuf</i> (EF-1)
	1272	<i>Exophiala moniliae</i>	This patent	<i>tuf</i> (EF-1)
	1273	<i>Hortaea wernickii</i>	This patent	<i>tuf</i> (EF-1)
	1274	<i>Fusarium solani</i>	This patent	<i>tuf</i> (EF-1)
45	1275	<i>Aureobasidium pullulans</i>	This patent	<i>tuf</i> (EF-1)
	1276	<i>Blastomyces dermatitidis</i>	This patent	<i>tuf</i> (EF-1)
	1277	<i>Exophiala dermatitidis</i>	This patent	<i>tuf</i> (EF-1)
	1278	<i>Fusarium moniliforme</i>	This patent	<i>tuf</i> (EF-1)
	1279	<i>Aspergillus terreus</i>	This patent	<i>tuf</i> (EF-1)
50	1280	<i>Aspergillus fumigatus</i>	This patent	<i>tuf</i> (EF-1)
	1281	<i>Cryptococcus laurentii</i>	This patent	<i>tuf</i> (EF-1)
	1282	<i>Emmonsia parva</i>	This patent	<i>tuf</i> (EF-1)
	1283	<i>Fusarium solani</i>	This patent	<i>tuf</i> (EF-1)
	1284	<i>Sporothrix schenckii</i>	This patent	<i>tuf</i> (EF-1)
55	1285	<i>Aspergillus nidulans</i>	This patent	<i>tuf</i> (EF-1)
	1286	<i>Cladophialophora carrionii</i>	This patent	<i>tuf</i> (EF-1)
	1287	<i>Exserohilum rostratum</i>	This patent	<i>tuf</i> (EF-1)
	1288	<i>Bacillus thuringiensis</i>	This patent	<i>recA</i>
	1289	<i>Bacillus thuringiensis</i>	This patent	<i>recA</i>
60	1299	<i>Staphylococcus aureus</i>	Database	<i>gyrA</i>
	1300	<i>Escherichia coli</i>	Database	<i>gyrA</i>
	1307	<i>Staphylococcus aureus</i>	Database	<i>gyrB</i>
	1320	<i>Escherichia coli</i>	Database	<i>parC</i> (<i>grlA</i>)
	1321	<i>Staphylococcus aureus</i>	Database	<i>parC</i> (<i>grlA</i>)
65	1328	<i>Staphylococcus aureus</i>	Database	<i>parE</i> (<i>grlB</i>)

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1348 unidentified bacterium	Database	<i>aac2Ia</i>
	1351 <i>Pseudomonas aeruginosa</i>	Database	<i>aac3Ib</i>
	1356 <i>Serratia marcescens</i>	Database	<i>aac3IIb</i>
	1361 <i>Escherichia coli</i>	Database	<i>aac3IVa</i>
	1366 <i>Enterobacter cloacae</i>	Database	<i>aac3VIa</i>
10	1371 <i>Citrobacter koseri</i>	Database	<i>aac6Ia</i>
	1376 <i>Serratia marcescens</i>	Database	<i>aac6Ic</i>
	1381 <i>Escherichia coli</i>	Database	<i>ant3Ia</i>
	1386 <i>Staphylococcus aureus</i>	Database	<i>ant4Ia</i>
	1391 <i>Escherichia coli</i>	Database	<i>aph3Ia</i>
15	1396 <i>Escherichia coli</i>	Database	<i>aph3IIa</i>
	1401 <i>Enterococcus faecalis</i>	Database	<i>aph3IIIa</i>
	1406 <i>Acinetobacter baumannii</i>	Database	<i>aph3VIa</i>
	1411 <i>Pseudomonas aeruginosa</i>	Database	<i>blaCARB</i>
	1416 <i>Klebsiella pneumoniae</i>	Database	<i>blaCMY-2</i>
20	1423 <i>Escherichia coli</i>	Database	<i>blaCTX-M-1</i>
	1428 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	Database	<i>blaCTX-M-2</i>
	1433 <i>Pseudomonas aeruginosa</i>	Database	<i>blaIMP</i>
	1438 <i>Escherichia coli</i>	Database	<i>blaOXA2</i>
	1439 <i>Pseudomonas aeruginosa</i>	Database	<i>blaOXA10</i>
25	1442 <i>Pseudomonas aeruginosa</i>	Database	<i>blaPER1</i>
	1445 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	Database	<i>blaPER2</i>
	1452 <i>Staphylococcus epidermidis</i>	Database	<i>dfrA</i>
	1461 <i>Escherichia coli</i>	Database	<i>dhfrIa</i>
	1470 <i>Escherichia coli</i>	Database	<i>dhfrIb</i>
30	1475 <i>Escherichia coli</i>	Database	<i>dhfrV</i>
	1480 <i>Proteus mirabilis</i>	Database	<i>dhfrVI</i>
	1489 <i>Escherichia coli</i>	Database	<i>dhfrVII</i>
	1494 <i>Escherichia coli</i>	Database	<i>dhfrVIII</i>
	1499 <i>Escherichia coli</i>	Database	<i>dhfrIX</i>
35	1504 <i>Escherichia coli</i>	Database	<i>dhfrXII</i>
	1507 <i>Escherichia coli</i>	Database	<i>dhfrXIII</i>
	1512 <i>Escherichia coli</i>	Database	<i>dhfrXV</i>
	1517 <i>Escherichia coli</i>	Database	<i>dhfrXVII</i>
	1518 <i>Acinetobacter lwoffii</i>	This patent	<i>fusA</i>
40	1519 <i>Acinetobacter lwoffii</i>	This patent	<i>fusA-tuf</i> spacer
	1520 <i>Acinetobacter lwoffii</i>	This patent	<i>tuf</i>
	1521 <i>Haemophilus influenzae</i>	This patent	<i>fusA</i>
	1522 <i>Haemophilus influenzae</i>	This patent	<i>fusA-tuf</i> spacer
	1523 <i>Haemophilus influenzae</i>	This patent	<i>tuf</i>
45	1524 <i>Proteus mirabilis</i>	This patent	<i>fusA</i>
	1525 <i>Proteus mirabilis</i>	This patent	<i>fusA-tuf</i> spacer
	1526 <i>Proteus mirabilis</i>	This patent	<i>tuf</i>
	1527 <i>Campylobacter curvus</i>	This patent	<i>atpD</i>
	1530 <i>Escherichia coli</i>	Database	<i>ereA</i>
50	1535 <i>Escherichia coli</i>	Database	<i>ereB</i>
	1540 <i>Staphylococcus haemolyticus</i>	Database	<i>linA</i>
	1545 <i>Enterococcus faecium</i>	Database	<i>linB</i>
	1548 <i>Streptococcus pyogenes</i>	Database	<i>mefA</i>
	1551 <i>Streptococcus pneumoniae</i>	Database	<i>mefE</i>
55	1560 <i>Escherichia coli</i>	Database	<i>mphA</i>
	1561 <i>Candida albicans</i>	This patent	<i>tuf</i> (EF-1)
	1562 <i>Candida dubliniensis</i>	This patent	<i>tuf</i> (EF-1)
	1563 <i>Candida famata</i>	This patent	<i>tuf</i> (EF-1)
	1564 <i>Candida glabrata</i>	This patent	<i>tuf</i> (EF-1)
60	1565 <i>Candida guilliermondii</i>	This patent	<i>tuf</i> (EF-1)
	1566 <i>Candida haemulonii</i>	This patent	<i>tuf</i> (EF-1)
	1567 <i>Candida kefyr</i>	This patent	<i>tuf</i> (EF-1)
	1568 <i>Candida lusitanae</i>	This patent	<i>tuf</i> (EF-1)

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1569 <i>Candida sphaerica</i>	This patent	<i>tuf</i> (EF-1)
	1570 <i>Candida tropicalis</i>	This patent	<i>tuf</i> (EF-1)
	1571 <i>Candida viswanathii</i>	This patent	<i>tuf</i> (EF-1)
	1572 <i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	This patent	<i>tuf</i>
	1573 <i>Prevotella buccalis</i>	This patent	<i>tuf</i>
10	1574 <i>Succinivibrio dextrinosolvens</i>	This patent	<i>tuf</i>
	1575 <i>Tetragenococcus halophilus</i>	This patent	<i>tuf</i>
	1576 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>atpD</i>
	1577 <i>Campylobacter rectus</i>	This patent	<i>atpD</i>
	1578 <i>Enterococcus casseliflavus</i>	This patent	<i>fusA</i>
15	1579 <i>Enterococcus gallinarum</i>	This patent	<i>fusA</i>
	1580 <i>Streptococcus mitis</i>	This patent	<i>fusA</i>
	1585 <i>Enterococcus faecium</i>	Database	<i>satG</i>
	1590 Cloning vector pFW16	Database	<i>tetM</i>
	1594 <i>Enterococcus faecium</i>	Database	<i>vanD</i>
20	1599 <i>Enterococcus faecalis</i>	Database	<i>vanE</i>
	1600 <i>Campylobacter jejuni</i> subsp. <i>doylei</i>	This patent	<i>atpD</i>
	1601 <i>Enterococcus sulfureus</i>	This patent	<i>atpD</i>
	1602 <i>Enterococcus solitarius</i>	This patent	<i>atpD</i>
	1603 <i>Campylobacter sputorum</i> subsp. <i>sputorum</i>	This patent	<i>atpD</i>
25	1604 <i>Enterococcus pseudoavium</i>	This patent	<i>atpD</i>
	1607 <i>Klebsiella omithinolytica</i>	This patent	<i>gyrA</i>
	1608 <i>Klebsiella oxytoca</i>	This patent	<i>gyrA</i>
	1613 <i>Staphylococcus aureus</i>	Database	<i>vatB</i>
	1618 <i>Staphylococcus cohnii</i>	Database	<i>vatC</i>
30	1623 <i>Staphylococcus aureus</i>	Database	<i>vga</i>
	1628 <i>Staphylococcus aureus</i>	Database	<i>vgaB</i>
	1633 <i>Staphylococcus aureus</i>	Database	<i>vgb</i>
	1638 <i>Aspergillus fumigatus</i>	This patent	<i>atpD</i>
	1639 <i>Aspergillus fumigatus</i>	This patent	<i>atpD</i>
35	1640 <i>Bacillus mycoides</i>	This patent	<i>atpD</i>
	1641 <i>Bacillus mycoides</i>	This patent	<i>atpD</i>
	1642 <i>Bacillus mycoides</i>	This patent	<i>atpD</i>
	1643 <i>Bacillus pseudomycoides</i>	This patent	<i>atpD</i>
	1644 <i>Bacillus pseudomycoides</i>	This patent	<i>atpD</i>
40	1645 <i>Budvicia aquatica</i>	This patent	<i>atpD</i>
	1646 <i>Buttiauxella agrestis</i>	This patent	<i>atpD</i>
	1647 <i>Candida norvegica</i>	This patent	<i>atpD</i>
	1648 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1a</i>
	1649 <i>Campylobacter lari</i>	This patent	<i>atpD</i>
45	1650 <i>Coccidioides immitis</i>	This patent	<i>atpD</i>
	1651 <i>Emmonsia parva</i>	This patent	<i>atpD</i>
	1652 <i>Erwinia amylovora</i>	This patent	<i>atpD</i>
	1653 <i>Fonsecaea pedrosoi</i>	This patent	<i>atpD</i>
	1654 <i>Fusarium moniliforme</i>	This patent	<i>atpD</i>
50	1655 <i>Klebsiella oxytoca</i>	This patent	<i>atpD</i>
	1656 <i>Microsporium audouinii</i>	This patent	<i>atpD</i>
	1657 <i>Obesumbacterium proteus</i>	This patent	<i>atpD</i>
	1658 <i>Paracoccidioides brasiliensis</i>	This patent	<i>atpD</i>
	1659 <i>Plesiomonas shigelloides</i>	This patent	<i>atpD</i>
55	1660 <i>Shewanella putrefaciens</i>	This patent	<i>atpD</i>
	1662 <i>Campylobacter curvus</i>	This patent	<i>tuf</i>
	1663 <i>Campylobacter rectus</i>	This patent	<i>tuf</i>
	1664 <i>Fonsecaea pedrosoi</i>	This patent	<i>tuf</i>
	1666 <i>Microsporium audouinii</i>	This patent	<i>tuf</i>
60	1667 <i>Piedraia hortai</i>	This patent	<i>tuf</i>
	1668 <i>Escherichia coli</i>	Database	<i>tuf</i>
	1669 <i>Saksenaea vasiformis</i>	This patent	<i>tuf</i>
	1670 <i>Trichophyton tonsurans</i>	This patent	<i>tuf</i>
	1671 <i>Enterobacter aerogenes</i>	This patent	<i>atpD</i>
65	1672 <i>Bordetella pertussis</i>	Database	<i>atpD</i>
	1673 <i>Arcanobacterium haemolyticum</i>	This patent	<i>tuf</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

	SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1674	<i>Butyrivibrio fibrisolvens</i>	This patent	<i>tuf</i>
	1675	<i>Campylobacter jejuni</i> subsp. <i>doylei</i>	This patent	<i>tuf</i>
	1676	<i>Campylobacter lari</i>	This patent	<i>tuf</i>
	1677	<i>Campylobacter sputorum</i> subsp. <i>sputorum</i>	This patent	<i>tuf</i>
	1678	<i>Campylobacter upsaliensis</i>	This patent	<i>tuf</i>
10	1679	<i>Globicatella sanguis</i>	This patent	<i>tuf</i>
	1680	<i>Lactobacillus acidophilus</i>	This patent	<i>tuf</i>
	1681	<i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>	This patent	<i>tuf</i>
	1682	<i>Prevotella buccalis</i>	This patent	<i>tuf</i>
	1683	<i>Ruminococcus bromii</i>	This patent	<i>tuf</i>
15	1684	<i>Paracoccidioides brasiliensis</i>	This patent	<i>atpD</i>
	1685	<i>Candida norvegica</i>	This patent	<i>tuf</i> (EF-1)
	1686	<i>Aspergillus nidulans</i>	This patent	<i>tuf</i>
	1687	<i>Aspergillus terreus</i>	This patent	<i>tuf</i>
	1688	<i>Candida norvegica</i>	This patent	<i>tuf</i>
20	1689	<i>Candida parapsilosis</i>	This patent	<i>tuf</i>
	1702	<i>Streptococcus gordonii</i>	WO98/20157	<i>recA</i>
	1703	<i>Streptococcus mutans</i>	WO98/20157	<i>recA</i>
	1704	<i>Streptococcus pneumoniae</i>	WO98/20157	<i>recA</i>
	1705	<i>Streptococcus pyogenes</i>	WO98/20157	<i>recA</i>
25	1706	<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	WO98/20157	<i>recA</i>
	1707	<i>Escherichia coli</i>	WO98/20157	<i>oxa</i>
	1708	<i>Enterococcus faecalis</i>	WO98/20157	<i>blaZ</i>
	1709	<i>Pseudomonas aeruginosa</i>	WO98/20157	<i>aac6'-IIa</i>
	1710	<i>Staphylococcus aureus</i>	WO98/20157	<i>ermA</i>
30	1711	<i>Escherichia coli</i>	WO98/20157	<i>ermB</i>
	1712	<i>Staphylococcus aureus</i>	WO98/20157	<i>ermC</i>
	1713	<i>Enterococcus faecalis</i>	WO98/20157	<i>vanB</i>
	1714	<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>recA</i>
	1715	<i>Abiotrophia adiacens</i>	WO98/20157	<i>tuf</i>
35	1716	<i>Abiotrophia defectiva</i>	WO98/20157	<i>tuf</i>
	1717	<i>Corynebacterium accolens</i>	WO98/20157	<i>tuf</i>
	1718	<i>Corynebacterium genitalium</i>	WO98/20157	<i>tuf</i>
	1719	<i>Corynebacterium jeikeium</i>	WO98/20157	<i>tuf</i>
	1720	<i>Corynebacterium pseudodiphtheriticum</i>	WO98/20157	<i>tuf</i>
40	1721	<i>Corynebacterium striatum</i>	WO98/20157	<i>tuf</i>
	1722	<i>Enterococcus avium</i>	WO98/20157	<i>tuf</i>
	1723	<i>Gardnerella vaginalis</i>	WO98/20157	<i>tuf</i>
	1724	<i>Listeria innocua</i>	WO98/20157	<i>tuf</i>
	1725	<i>Listeria ivanovii</i>	WO98/20157	<i>tuf</i>
45	1726	<i>Listeria monocytogenes</i>	WO98/20157	<i>tuf</i>
	1727	<i>Listeria seeligeri</i>	WO98/20157	<i>tuf</i>
	1728	<i>Staphylococcus aureus</i>	WO98/20157	<i>tuf</i>
	1729	<i>Staphylococcus saprophyticus</i>	WO98/20157	<i>tuf</i>
	1730	<i>Staphylococcus simulans</i>	WO98/20157	<i>tuf</i>
50	1731	<i>Streptococcus agalactiae</i>	WO98/20157	<i>tuf</i>
	1732	<i>Streptococcus pneumoniae</i>	WO98/20157	<i>tuf</i>
	1733	<i>Streptococcus salivarius</i>	WO98/20157	<i>tuf</i>
	1734	<i>Agrobacterium radiobacter</i>	WO98/20157	<i>tuf</i>
	1735	<i>Bacillus subtilis</i>	WO98/20157	<i>tuf</i>
55	1736	<i>Bacteroides fragilis</i>	WO98/20157	<i>tuf</i>
	1737	<i>Borrelia burgdorferi</i>	WO98/20157	<i>tuf</i>
	1738	<i>Brevibacterium linens</i>	WO98/20157	<i>tuf</i>
	1739	<i>Chlamydia trachomatis</i>	WO98/20157	<i>tuf</i>
	1740	<i>Fibrobacter succinogenes</i>	WO98/20157	<i>tuf</i>
60	1741	<i>Flavobacterium ferrugineum</i>	WO98/20157	<i>tuf</i>
	1742	<i>Helicobacter pylori</i>	WO98/20157	<i>tuf</i>
	1743	<i>Micrococcus luteus</i>	WO98/20157	<i>tuf</i>
	1744	<i>Mycobacterium tuberculosis</i>	WO98/20157	<i>tuf</i>
	1745	<i>Mycoplasma genitalium</i>	WO98/20157	<i>tuf</i>
65	1746	<i>Neisseria gonorrhoeae</i>	WO98/20157	<i>tuf</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1747 <i>Rickettsia prowazekii</i>	WO98/20157	tuf
	1748 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i>	WO98/20157	tuf
	serotype Typhimurium		
	1749 <i>Shewanella putrefaciens</i>	WO98/20157	tuf
	1750 <i>Stigmatella aurantiaca</i>	WO98/20157	tuf
10	1751 <i>Thiomonas cuprina</i>	WO98/20157	tuf
	1752 <i>Treponema pallidum</i>	WO98/20157	tuf
	1753 <i>Ureaplasma urealyticum</i>	WO98/20157	tuf
	1754 <i>Wolinella succinogenes</i>	WO98/20157	tuf
	1755 <i>Burkholderia cepacia</i>	WO98/20157	tuf
15	1756 <i>Bacillus anthracis</i>	This patent	recA
	1757 <i>Bacillus anthracis</i>	This patent	recA
	1758 <i>Bacillus cereus</i>	This patent	recA
	1759 <i>Bacillus cereus</i>	This patent	recA
	1760 <i>Bacillus mycoides</i>	This patent	recA
20	1761 <i>Bacillus pseudomycoides</i>	This patent	recA
	1762 <i>Bacillus thuringiensis</i>	This patent	recA
	1763 <i>Bacillus thuringiensis</i>	This patent	recA
	1764 <i>Klebsiella oxytoca</i>	This patent	gyrA
	1765 <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	This patent	gyrA
25	1766 <i>Klebsiella planticola</i>	This patent	gyrA
	1767 <i>Klebsiella pneumoniae</i>	This patent	gyrA
	1768 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	gyrA
	1769 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	gyrA
	1770 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>	This patent	gyrA
30	1771 <i>Klebsiella terrigena</i>	This patent	gyrA
	1772 <i>Legionella pneumophila</i> subsp. <i>pneumophila</i>	This patent	gyrA
	1773 <i>Proteus mirabilis</i>	This patent	gyrA
	1774 <i>Providencia rettgeri</i>	This patent	gyrA
	1775 <i>Proteus vulgaris</i>	This patent	gyrA
35	1776 <i>Yersinia enterocolitica</i>	This patent	gyrA
	1777 <i>Klebsiella oxytoca</i>	This patent	parC (grlA)
	1778 <i>Klebsiella oxytoca</i>	This patent	parC (grlA)
	1779 <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	This patent	parC (grlA)
	1780 <i>Klebsiella planticola</i>	This patent	parC (grlA)
40	1781 <i>Klebsiella pneumoniae</i>	This patent	parC (grlA)
	1782 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	parC (grlA)
	1783 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	parC (grlA)
	1784 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>	This patent	parC (grlA)
	1785 <i>Klebsiella terrigena</i>	This patent	parC (grlA)
45	1786 <i>Bacillus cereus</i>	This patent	fusA
	1787 <i>Bacillus cereus</i>	This patent	fusA
	1788 <i>Bacillus anthracis</i>	This patent	fusA
	1789 <i>Bacillus cereus</i>	This patent	fusA
	1790 <i>Bacillus anthracis</i>	This patent	fusA
50	1791 <i>Bacillus pseudomycoides</i>	This patent	fusA
	1792 <i>Bacillus cereus</i>	This patent	fusA
	1793 <i>Bacillus anthracis</i>	This patent	fusA
	1794 <i>Bacillus cereus</i>	This patent	fusA
	1795 <i>Bacillus weihenstephanensis</i>	This patent	fusA
55	1796 <i>Bacillus mycoides</i>	This patent	fusA
	1797 <i>Bacillus thuringiensis</i>	This patent	fusA
	1798 <i>Bacillus weihenstephanensis</i>	This patent	fusA-tuf spacer
	1799 <i>Bacillus thuringiensis</i>	This patent	fusA-tuf spacer
	1800 <i>Bacillus anthracis</i>	This patent	fusA-tuf spacer
60	1801 <i>Bacillus pseudomycoides</i>	This patent	fusA-tuf spacer
	1802 <i>Bacillus anthracis</i>	This patent	fusA-tuf spacer
	1803 <i>Bacillus cereus</i>	This patent	fusA-tuf spacer
	1804 <i>Bacillus cereus</i>	This patent	fusA-tuf spacer
	1805 <i>Bacillus mycoides</i>	This patent	fusA-tuf spacer
65	1806 <i>Bacillus cereus</i>	This patent	fusA-tuf spacer

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1807 <i>Bacillus cereus</i>	This patent	<i>fusA-tuf</i> spacer
	1808 <i>Bacillus cereus</i>	This patent	<i>fusA-tuf</i> spacer
	1809 <i>Bacillus anthracis</i>	This patent	<i>fusA-tuf</i> spacer
	1810 <i>Bacillus mycoides</i>	This patent	<i>tuf</i>
	1811 <i>Bacillus thuringiensis</i>	This patent	<i>tuf</i>
10	1812 <i>Bacillus cereus</i>	This patent	<i>tuf</i>
	1813 <i>Bacillus weihenstephanensis</i>	This patent	<i>tuf</i>
	1814 <i>Bacillus anthracis</i>	This patent	<i>tuf</i>
	1815 <i>Bacillus cereus</i>	This patent	<i>tuf</i>
	1816 <i>Bacillus cereus</i>	This patent	<i>tuf</i>
15	1817 <i>Bacillus anthracis</i>	This patent	<i>tuf</i>
	1818 <i>Bacillus cereus</i>	This patent	<i>tuf</i>
	1819 <i>Bacillus anthracis</i>	This patent	<i>tuf</i>
	1820 <i>Bacillus pseudomycoloides</i>	This patent	<i>tuf</i>
	1821 <i>Bacillus cereus</i>	This patent	<i>tuf</i>
20	1822 <i>Streptococcus oralis</i>	This patent	<i>fusA</i>
	1823 <i>Budvicia aquatica</i>	This patent	<i>fusA</i>
	1824 <i>Buttiauxella agrestis</i>	This patent	<i>fusA</i>
	1825 <i>Klebsiella oxytoca</i>	This patent	<i>fusA</i>
	1826 <i>Plesiomonas shigelloides</i>	This patent	<i>fusA</i>
25	1827 <i>Shewanella putrefaciens</i>	This patent	<i>fusA</i>
	1828 <i>Obesumbacterium proteus</i>	This patent	<i>fusA</i>
	1829 <i>Klebsiella oxytoca</i>	This patent	<i>fusA-tuf</i> spacer
	1830 <i>Budvicia aquatica</i>	This patent	<i>fusA-tuf</i> spacer
	1831 <i>Plesiomonas shigelloides</i>	This patent	<i>fusA-tuf</i> spacer
30	1832 <i>Obesumbacterium proteus</i>	This patent	<i>fusA-tuf</i> spacer
	1833 <i>Shewanella putrefaciens</i>	This patent	<i>fusA-tuf</i> spacer
	1834 <i>Buttiauxella agrestis</i>	This patent	<i>fusA-tuf</i> spacer
	1835 <i>Campylobacter coli</i>	This patent	<i>tuf</i>
	1836 <i>Campylobacter fetus</i> subsp. <i>fetus</i>	This patent	<i>tuf</i>
35	1837 <i>Campylobacter fetus</i> subsp. <i>venerealis</i>	This patent	<i>tuf</i>
	1838 <i>Buttiauxella agrestis</i>	This patent	<i>tuf</i>
	1839 <i>Klebsiella oxytoca</i>	This patent	<i>tuf</i>
	1840 <i>Plesiomonas shigelloides</i>	This patent	<i>tuf</i>
	1841 <i>Shewanella putrefaciens</i>	This patent	<i>tuf</i>
40	1842 <i>Obesumbacterium proteus</i>	This patent	<i>tuf</i>
	1843 <i>Budvicia aquatica</i>	This patent	<i>tuf</i>
	1844 <i>Abiotrophia adiacens</i>	This patent	<i>atpD</i>
	1845 <i>Arcanobacterium haemolyticum</i>	This patent	<i>atpD</i>
	1846 <i>Basidiobolus ranarum</i>	This patent	<i>atpD</i>
45	1847 <i>Blastomyces dermatitidis</i>	This patent	<i>atpD</i>
	1848 <i>Blastomyces dermatitidis</i>	This patent	<i>atpD</i>
	1849 <i>Campylobacter coli</i>	This patent	<i>atpD</i>
	1850 <i>Campylobacter fetus</i> subsp. <i>fetus</i>	This patent	<i>atpD</i>
	1851 <i>Campylobacter fetus</i> subsp. <i>venerealis</i>	This patent	<i>atpD</i>
50	1852 <i>Campylobacter gracilis</i>	This patent	<i>atpD</i>
	1853 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>atpD</i>
	1854 <i>Enterococcus cecorum</i>	This patent	<i>atpD</i>
	1855 <i>Enterococcus columbae</i>	This patent	<i>atpD</i>
	1856 <i>Enterococcus dispar</i>	This patent	<i>atpD</i>
55	1857 <i>Enterococcus malodoratus</i>	This patent	<i>atpD</i>
	1858 <i>Enterococcus mundtii</i>	This patent	<i>atpD</i>
	1859 <i>Enterococcus raffinosus</i>	This patent	<i>atpD</i>
	1860 <i>Globicatella sanguis</i>	This patent	<i>atpD</i>
	1861 <i>Lactococcus garvieae</i>	This patent	<i>atpD</i>
60	1862 <i>Lactococcus lactis</i>	This patent	<i>atpD</i>
	1863 <i>Listeria ivanovii</i>	This patent	<i>atpD</i>
	1864 <i>Succinivibrio dextrinosolvens</i>	This patent	<i>atpD</i>
	1865 <i>Tetragenococcus halophilus</i>	This patent	<i>atpD</i>
	1866 <i>Campylobacter fetus</i> subsp. <i>fetus</i>	This patent	<i>recA</i>
65	1867 <i>Campylobacter fetus</i> subsp. <i>venerealis</i>	This patent	<i>recA</i>
	1868 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>recA</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	1869 <i>Enterococcus avium</i>	This patent	<i>recA</i>
	1870 <i>Enterococcus faecium</i>	This patent	<i>recA</i>
	1871 <i>Listeria monocytogenes</i>	This patent	<i>recA</i>
	1872 <i>Streptococcus mitis</i>	This patent	<i>recA</i>
	1873 <i>Streptococcus oralis</i>	This patent	<i>recA</i>
10	1874 <i>Aspergillus fumigatus</i>	This patent	<i>tuf</i> (M)
	1875 <i>Aspergillus versicolor</i>	This patent	<i>tuf</i> (M)
	1876 <i>Basidiobolus ranarum</i>	This patent	<i>tuf</i> (M)
	1877 <i>Campylobacter gracilis</i>	This patent	<i>tuf</i>
	1878 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>tuf</i>
15	1879 <i>Coccidioides immitis</i>	This patent	<i>tuf</i> (M)
	1880 <i>Erwinia amylovora</i>	This patent	<i>tuf</i>
	1881 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	This patent	<i>tuf</i>
20	1899 <i>Klebsiella pneumoniae</i>	Database	<i>blaSHV</i>
	1900 <i>Klebsiella pneumoniae</i>	Database	<i>blaSHV</i>
	1901 <i>Escherichia coli</i>	Database	<i>blaSHV</i>
	1902 <i>Klebsiella pneumoniae</i>	Database	<i>blaSHV</i>
	1903 <i>Klebsiella pneumoniae</i>	Database	<i>blaSHV</i>
25	1904 <i>Escherichia coli</i>	Database	<i>blaSHV</i>
	1905 <i>Pseudomonas aeruginosa</i>	Database	<i>blaSHV</i>
	1927 <i>Neisseria meningitidis</i>	Database	<i>blaTEM</i>
	1928 <i>Escherichia coli</i>	Database	<i>blaTEM</i>
	1929 <i>Klebsiella oxytoca</i>	Database	<i>blaTEM</i>
30	1930 <i>Escherichia coli</i>	Database	<i>blaTEM</i>
	1931 <i>Escherichia coli</i>	Database	<i>blaTEM</i>
	1932 <i>Escherichia coli</i>	Database	<i>blaTEM</i>
	1933 <i>Escherichia coli</i>	Database	<i>blaTEM</i>
	1954 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	Database	<i>gyrA</i>
35	1956 <i>Candida inconspicua</i>	This patent	<i>tuf</i> (M)
	1957 <i>Candida utilis</i>	This patent	<i>tuf</i> (M)
	1958 <i>Candida zeylanoides</i>	This patent	<i>tuf</i> (M)
	1959 <i>Candida catenulata</i>	This patent	<i>tuf</i> (M)
	1960 <i>Candida krusei</i>	This patent	<i>tuf</i> (M)
40	1965 Plasmid pGS05	Database	<i>sullI</i>
	1970 Transposon Tn10	Database	<i>tetB</i>
	1985 <i>Cryptococcus neoformans</i>	Database	<i>tuf</i> (EF-1)
	1986 <i>Cryptococcus neoformans</i>	Database	<i>tuf</i> (EF-1)
	1987 <i>Saccharomyces cerevisiae</i>	Database	<i>tuf</i> (EF-1)
45	1988 <i>Saccharomyces cerevisiae</i>	Database	<i>tuf</i> (EF-1)
	1989 <i>Eremothecium gossypii</i>	Database	<i>tuf</i> (EF-1)
	1990 <i>Eremothecium gossypii</i>	Database	<i>tuf</i> (EF-1)
	1991 <i>Aspergillus oryzae</i>	Database	<i>tuf</i> (EF-1)
	1992 <i>Aureobasidium pullulans</i>	Database	<i>tuf</i> (EF-1)
50	1993 <i>Histoplasma capsulatum</i>	Database	<i>tuf</i> (EF-1)
	1994 <i>Neurospora crassa</i>	Database	<i>tuf</i> (EF-1)
	1995 <i>Podospora anserina</i>	Database	<i>tuf</i> (EF-1)
	1996 <i>Podospora curvicolle</i>	Database	<i>tuf</i> (EF-1)
	1997 <i>Sordaria macrospora</i>	Database	<i>tuf</i> (EF-1)
55	1998 <i>Trichoderma reesei</i>	Database	<i>tuf</i> (EF-1)
	2004 <i>Candida albicans</i>	Database	<i>tuf</i> (M)
	2005 <i>Schizosaccharomyces pombe</i>	Database	<i>tuf</i> (M)
	2010 <i>Klebsiella pneumoniae</i>	Database	<i>blaTEM</i>
	2011 <i>Klebsiella pneumoniae</i>	Database	<i>blaTEM</i>
60	2013 <i>Kluyvera ascorbata</i>	This patent	<i>gyrA</i>
	2014 <i>Kluyvera georgiana</i>	This patent	<i>gyrA</i>
	2047 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2048 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2049 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	2050 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2051 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2052 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2053 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1A</i>
	2054 <i>Streptococcus pneumoniae</i>	Database	<i>gyrA</i>
10	2055 <i>Streptococcus pneumoniae</i>	Database	<i>parC</i>
	2056 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2057 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2058 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2059 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
15	2060 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2061 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2062 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2063 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
	2064 <i>Streptococcus pneumoniae</i>	This patent	<i>pbp1A</i>
20	2072 <i>Mycobacterium tuberculosis</i>	Database	<i>rpoB</i>
	2097 <i>Mycoplasma pneumoniae</i>	Database	<i>tuf</i>
	2101 <i>Mycobacterium tuberculosis</i>	Database	<i>inhA</i>
	2105 <i>Mycobacterium tuberculosis</i>	Database	<i>embB</i>
	2129 <i>Clostridium difficile</i>	Database	<i>cdtA</i>
25	2130 <i>Clostridium difficile</i>	Database	<i>cdtB</i>
	2137 <i>Pseudomonas putida</i>	Genome project	<i>tuf</i>
	2138 <i>Pseudomonas aeruginosa</i>	Genome project	<i>tuf</i>
	2139 <i>Campylobacter jejuni</i>	Database	<i>atpD</i>
	2140 <i>Streptococcus pneumoniae</i>	Database	<i>pbp1a</i>
30	2144 <i>Staphylococcus aureus</i>	Database	<i>mupA</i>
	2147 <i>Escherichia coli</i>	Database	<i>catI</i>
	2150 <i>Escherichia coli</i>	Database	<i>catII</i>
	2153 <i>Shigella flexneri</i>	Database	<i>catIII</i>
	2156 <i>Clostridium perfringens</i>	Database	<i>catP</i>
35	2159 <i>Staphylococcus aureus</i>	Database	<i>cat</i>
	2162 <i>Staphylococcus aureus</i>	Database	<i>cat</i>
	2165 <i>Salmonella typhimurium</i>	Database	<i>ppfl</i> -like
	2183 <i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	This patent	<i>tuf</i>
	2184 <i>Campylobacter coli</i>	This patent	<i>fusA</i>
40	2185 <i>Succinivibrio dextrinosolvens</i>	This patent	<i>tuf</i>
	2186 <i>Tetragenococcus halophilus</i>	This patent	<i>tuf</i>
	2187 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>fusA</i>
	2188 <i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	This patent	<i>fusA</i>
	2189 <i>Leishmania guyanensis</i>	This patent	<i>atpD</i>
45	2190 <i>Trypanosoma brucei brucei</i>	This patent	<i>atpD</i>
	2191 <i>Aspergillus nidulans</i>	This patent	<i>atpD</i>
	2192 <i>Leishmania panamensis</i>	This patent	<i>atpD</i>
	2193 <i>Aspergillus nidulans</i>	This patent	<i>tuf</i> (M)
	2194 <i>Aureobasidium pullulans</i>	This patent	<i>tuf</i> (M)
50	2195 <i>Emmonsia parva</i>	This patent	<i>tuf</i> (M)
	2196 <i>Exserohilum rostratum</i>	This patent	<i>tuf</i> (M)
	2197 <i>Fusarium moniliforme</i>	This patent	<i>tuf</i> (M)
	2198 <i>Fusarium solani</i>	This patent	<i>tuf</i> (M)
	2199 <i>Histoplasma capsulatum</i>	This patent	<i>tuf</i> (M)
55	2200 <i>Kocuria kristinae</i>	This patent	<i>tuf</i>
	2201 <i>Vibrio mimicus</i>	This patent	<i>tuf</i>
	2202 <i>Citrobacter freundii</i>	This patent	<i>recA</i>
	2203 <i>Clostridium botulinum</i>	This patent	<i>recA</i>
	2204 <i>Francisella tularensis</i>	This patent	<i>recA</i>
60	2205 <i>Peptostreptococcus anaerobius</i>	This patent	<i>recA</i>
	2206 <i>Peptostreptococcus asaccharolyticus</i>	This patent	<i>recA</i>
	2207 <i>Providencia stuartii</i>	This patent	<i>recA</i>

Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	2208 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Paratyphi A	This patent	<i>recA</i>
	2209 <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	This patent	<i>recA</i>
	2210 <i>Staphylococcus saprophyticus</i>	This patent	<i>recA</i>
10	2211 <i>Yersinia pseudotuberculosis</i>	This patent	<i>recA</i>
	2212 <i>Zoogloea ramigera</i>	This patent	<i>recA</i>
	2214 <i>Abiotrophia adiacens</i>	This patent	<i>fusA</i>
	2215 <i>Acinetobacter baumannii</i>	This patent	<i>fusA</i>
	2216 <i>Actinomyces meyeri</i>	This patent	<i>fusA</i>
15	2217 <i>Clostridium difficile</i>	This patent	<i>fusA</i>
	2218 <i>Corynebacterium diphtheriae</i>	This patent	<i>fusA</i>
	2219 <i>Enterobacter cloacae</i>	This patent	<i>fusA</i>
	2220 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	<i>fusA</i>
	2221 <i>Listeria monocytogenes</i>	This patent	<i>fusA</i>
20	2222 <i>Mycobacterium avium</i>	This patent	<i>fusA</i>
	2223 <i>Mycobacterium gordonae</i>	This patent	<i>fusA</i>
	2224 <i>Mycobacterium kansasii</i>	This patent	<i>fusA</i>
	2225 <i>Mycobacterium terrae</i>	This patent	<i>fusA</i>
	2226 <i>Neisseria polysaccharea</i>	This patent	<i>fusA</i>
25	2227 <i>Staphylococcus epidermidis</i>	This patent	<i>fusA</i>
	2228 <i>Staphylococcus haemolyticus</i>	This patent	<i>fusA</i>
	2229 <i>Succinivibrio dextrinosolvens</i>	This patent	<i>fusA</i>
	2230 <i>Tetragenococcus halophilus</i>	This patent	<i>fusA</i>
	2231 <i>Veillonella parvula</i>	This patent	<i>fusA</i>
30	2232 <i>Yersinia pseudotuberculosis</i>	This patent	<i>fusA</i>
	2233 <i>Zoogloea ramigera</i>	This patent	<i>fusA</i>
	2234 <i>Aeromonas hydrophila</i>	This patent	<i>fusA</i>
	2235 <i>Abiotrophia adiacens</i>	This patent	<i>fusA-tuf</i> spacer
	2236 <i>Acinetobacter baumannii</i>	This patent	<i>fusA-tuf</i> spacer
35	2237 <i>Actinomyces meyeri</i>	This patent	<i>fusA-tuf</i> spacer
	2238 <i>Clostridium difficile</i>	This patent	<i>fusA-tuf</i> spacer
	2239 <i>Corynebacterium diphtheriae</i>	This patent	<i>fusA-tuf</i> spacer
	2240 <i>Enterobacter cloacae</i>	This patent	<i>fusA-tuf</i> spacer
	2241 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	<i>fusA-tuf</i> spacer
40	2242 <i>Listeria monocytogenes</i>	This patent	<i>fusA-tuf</i> spacer
	2243 <i>Mycobacterium avium</i>	This patent	<i>fusA-tuf</i> spacer
	2244 <i>Mycobacterium gordonae</i>	This patent	<i>fusA-tuf</i> spacer
	2245 <i>Mycobacterium kansasii</i>	This patent	<i>fusA-tuf</i> spacer
	2246 <i>Mycobacterium terrae</i>	This patent	<i>fusA-tuf</i> spacer
45	2247 <i>Neisseria polysaccharea</i>	This patent	<i>fusA-tuf</i> spacer
	2248 <i>Staphylococcus epidermidis</i>	This patent	<i>fusA-tuf</i> spacer
	2249 <i>Staphylococcus haemolyticus</i>	This patent	<i>fusA-tuf</i> spacer
	2255 <i>Abiotrophia adiacens</i>	This patent	<i>tuf</i>
	2256 <i>Acinetobacter baumannii</i>	This patent	<i>tuf</i>
50	2257 <i>Actinomyces meyeri</i>	This patent	<i>tuf</i>
	2258 <i>Clostridium difficile</i>	This patent	<i>tuf</i>
	2259 <i>Corynebacterium diphtheriae</i>	This patent	<i>tuf</i>
	2260 <i>Enterobacter cloacae</i>	This patent	<i>tuf</i>
	2261 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	This patent	<i>tuf</i>
55	2262 <i>Listeria monocytogenes</i>	This patent	<i>tuf</i>
	2263 <i>Mycobacterium avium</i>	This patent	<i>tuf</i>
	2264 <i>Mycobacterium gordonae</i>	This patent	<i>tuf</i>
	2265 <i>Mycobacterium kansasii</i>	This patent	<i>tuf</i>
	2266 <i>Mycobacterium terrae</i>	This patent	<i>tuf</i>
60	2267 <i>Neisseria polysaccharea</i>	This patent	<i>tuf</i>
	2268 <i>Staphylococcus epidermidis</i>	This patent	<i>tuf</i>
	2269 <i>Staphylococcus haemolyticus</i>	This patent	<i>tuf</i>
	2270 <i>Aeromonas hydrophila</i>	This patent	<i>tuf</i>
	2271 <i>Bilophila wadsworthia</i>	This patent	<i>tuf</i>
65	2272 <i>Brevundimonas diminuta</i>	This patent	<i>tuf</i>
	2273 <i>Streptococcus mitis</i>	This patent	<i>pbp1a</i>

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Table 7. Origin of the nucleic acids and/or sequences in the sequence listing (continued).

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
5	2274 <i>Streptococcus mitis</i>	This patent	<i>pbp1a</i>
	2275 <i>Streptococcus mitis</i>	This patent	<i>pbp1a</i>
	2276 <i>Streptococcus oralis</i>	This patent	<i>pbp1a</i>
	2277 <i>Escherichia coli</i>	This patent	<i>gyrA</i>
	2278 <i>Escherichia coli</i>	This patent	<i>gyrA</i>
10	2279 <i>Escherichia coli</i>	This patent	<i>gyrA</i>
	2280 <i>Escherichia coli</i>	This patent	<i>gyrA</i>
	2288 <i>Enterococcus faecium</i>	Database	<i>ddl</i>
	2293 <i>Enterococcus faecium</i>	Database	<i>vanA</i>
15	2296 <i>Enterococcus faecalis</i>	Database	<i>vanB</i>

* *tuf* indicates *tuf* sequences, *tuf* (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu, *tuf* (EF-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1 α), *tuf* (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin.

fusA indicates *fusA* sequences; *fusA-tuf* spacer indicates the intergenic region between *fusA* and *tuf*.

atpD indicates *atpD* sequences of the F-type, *atpD* (V) indicates *atpD* sequences of the V-type.

recA indicates *recA* sequences, *recA*(Rad51) indicates *rad51* sequences or homologs and *recA*(Dmc1) indicates *dmc1* sequences or homologs.

Table 8. Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers derived from *tuf* sequences.

	Strain	Reference number	Strain	Reference number
5	<i>Streptococcus acidominimus</i>	ATCC 51726	<i>Bacteroides caccae</i>	ATCC 43185
	<i>Streptococcus agalactiae</i>	ATCC 12403	<i>Bacteroides vulgatus</i>	ATCC 8482
	<i>Streptococcus agalactiae</i>	ATCC 12973	<i>Bacteroides fragilis</i>	ATCC 25285
10	<i>Streptococcus agalactiae</i>	ATCC 13813	<i>Candida albicans</i>	ATCC 11006
	<i>Streptococcus agalactiae</i>	ATCC 27591	<i>Clostridium innoculum</i>	ATCC 14501
	<i>Streptococcus agalactiae</i>	CDCs 1073	<i>Clostridium ramosum</i>	ATCC 25582
	<i>Streptococcus anginosus</i>	ATCC 27335	<i>Lactobacillus casei</i> subsp. <i>casei</i>	ATCC 393
	<i>Streptococcus anginosus</i>	ATCC 33397	<i>Clostridium septicum</i>	ATCC 12464
15	<i>Streptococcus bovis</i>	ATCC 33317	<i>Corynebacterium cervicis</i>	NCTC 10604
	<i>Streptococcus anginosus</i>	ATCC 27823	<i>Corynebacterium genitalium</i>	ATCC 33031
	<i>Streptococcus cricetus</i>	ATCC 19642	<i>Corynebacterium urealyticum</i>	ATCC 43042
	<i>Streptococcus cristatus</i>	ATCC 51100	<i>Enterococcus faecalis</i>	ATCC 29212
	<i>Streptococcus downei</i>	ATCC 33748	<i>Enterococcus faecium</i>	ATCC 19434
20	<i>Streptococcus dysgalactiae</i>	ATCC 43078	<i>Eubacterium lentum</i>	ATCC 43055
	<i>Streptococcus equi</i> subsp. <i>equi</i>	ATCC 9528	<i>Eubacterium nodutum</i>	ATCC 33099
	<i>Streptococcus ferus</i>	ATCC 33477	<i>Gardnerella vaginalis</i>	ATCC 14018
	<i>Streptococcus gordonii</i>	ATCC 10558	<i>Lactobacillus acidophilus</i>	ATCC 4356
	<i>Streptococcus macacae</i>	ATCC 35911	<i>Lactobacillus crispatus</i>	ATCC 33820
25	<i>Streptococcus mitis</i>	ATCC 49456	<i>Lactobacillus gasseri</i>	ATCC 33323
	<i>Streptococcus mutans</i>	ATCC 25175	<i>Lactobacillus johnsonii</i>	ATCC 33200
	<i>Streptococcus oralis</i>	ATCC 35037	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	ATCC 19435
	<i>Streptococcus parasanguinis</i>	ATCC 15912	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	ATCC 11454
	<i>Streptococcus parauberis</i>	DSM 6631	<i>Listeria innocua</i>	ATCC 33090
30	<i>Streptococcus pneumoniae</i>	ATCC 27336	<i>Micrococcus luteus</i>	ATCC 9341
	<i>Streptococcus pyogenes</i>	ATCC 19615	<i>Escherichia coli</i>	ATCC 25922
	<i>Streptococcus rattii</i>	ATCC 19645	<i>Micrococcus lylae</i>	ATCC 27566
	<i>Streptococcus salivarius</i>	ATCC 7073	<i>Porphyromonas asaccharolytica</i>	ATCC 25260
	<i>Streptococcus sanguinis</i>	ATCC 10556	<i>Prevotella corporis</i>	ATCC 33547
35	<i>Streptococcus sobrinus</i>	ATCC 27352	<i>Prevotella melanogenica</i>	ATCC 25845
	<i>Streptococcus suis</i>	ATCC 43765	<i>Staphylococcus aureus</i>	ATCC 13301
	<i>Streptococcus uberis</i>	ATCC 19436	<i>Staphylococcus epidermidis</i>	ATCC 14990
	<i>Streptococcus vestibularis</i>	ATCC 49124	<i>Staphylococcus saprophyticus</i>	ATCC 15305

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Table 9. Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers derived from *atpD* sequences.

	Strain	Reference number	Strain	Reference number
5	<i>Streptococcus acidominimus</i>	ATCC 51726	<i>Streptococcus gordonii</i>	ATCC 10558
	<i>Streptococcus agalactiae</i>	ATCC 12400	<i>Streptococcus macacae</i>	ATCC 35911
	<i>Streptococcus agalactiae</i>	ATCC 12403	<i>Streptococcus mitis</i>	ATCC 49456
10	<i>Streptococcus agalactiae</i>	ATCC 12973	<i>Streptococcus mutans</i>	ATCC 25175
	<i>Streptococcus agalactiae</i>	ATCC 13813	<i>Streptococcus oralis</i>	ATCC 35037
	<i>Streptococcus agalactiae</i>	ATCC 27591	<i>Streptococcus parasanguinis</i>	ATCC 15912
	<i>Streptococcus agalactiae</i>	CDCs-1073	<i>Streptococcus parauberis</i>	DSM 6631
	<i>Streptococcus anginosus</i>	ATCC 27335	<i>Streptococcus pneumoniae</i>	ATCC 27336
15	<i>Streptococcus anginosus</i>	ATCC 27823	<i>Streptococcus pyogenes</i>	ATCC 19615
	<i>Streptococcus bovis</i>	ATCC 33317	<i>Streptococcus rattii</i>	ATCC 19645
	<i>Streptococcus cricetus</i>	ATCC 19642	<i>Streptococcus salivarius</i>	ATCC 7073
	<i>Streptococcus cristatus</i>	ATCC 51100	<i>Streptococcus sanguinis</i>	ATCC 10556
	<i>Streptococcus downei</i>	ATCC 33748	<i>Streptococcus sobrinus</i>	ATCC 27352
20	<i>Streptococcus dysgalactiae</i>	ATCC 43078	<i>Streptococcus suis</i>	ATCC 43765
	<i>Streptococcus equi</i> subsp. <i>equi</i>	ATCC 9528	<i>Streptococcus uberis</i>	ATCC 19436
	<i>Streptococcus ferus</i>	ATCC 33477	<i>Streptococcus vestibularis</i>	ATCC 49124

Table 10. Bacterial species used to test the specificity of the *Enterococcus*-specific amplification primers derived from *tuf* sequences.

	Strain	Reference number	Strain	Reference number
5	Gram-positive species (n=74)			
	<i>Abiotrophia adiacens</i>	ATCC 49176	<i>Listeria innocua</i>	ATCC 33090
	<i>Abiotrophia defectiva</i>	ATCC 49175	<i>Listeria ivanovii</i>	ATCC 19119
	<i>Bacillus cereus</i>	ATCC 14579	<i>Listeria monocytogenes</i>	ATCC 15313
	<i>Bacillus subtilis</i>	ATCC 27370	<i>Listeria seeligeri</i>	ATCC 35967
10	<i>Bifidobacterium adolescentis</i>	ATCC 27534	<i>Micrococcus luteus</i>	ATCC 9341
	<i>Bifidobacterium breve</i>	ATCC 15700	<i>Pediococcus acidilacti</i>	ATCC 33314
	<i>Bifidobacterium dentium</i>	ATCC 27534	<i>Pediococcus pentosaceus</i>	ATCC 33316
	<i>Bifidobacterium longum</i>	ATCC 15707	<i>Peptococcus niger</i>	ATCC 27731
	<i>Clostridium perfringens</i>	ATCC 3124	<i>Peptostreptococcus anaerobius</i>	ATCC 27337
15	<i>Clostridium septicum</i>	ATCC 12464	<i>Peptostreptococcus indolicus</i>	ATCC 29247
	<i>Corynebacterium aquaticus</i>	ATCC 14665	<i>Peptostreptococcus micros</i>	ATCC 33270
	<i>Corynebacterium pseudodiphtheriticum</i>	ATCC 10700	<i>Propionibacterium acnes</i>	ATCC 6919
	<i>Enterococcus avium</i>	ATCC 14025	<i>Staphylococcus aureus</i>	ATCC 43300
20	<i>Enterococcus casseliflavus</i>	ATCC 25788	<i>Staphylococcus capitis</i>	ATCC 27840
	<i>Enterococcus cecorum</i>	ATCC 43199	<i>Staphylococcus epidermidis</i>	ATCC 14990
	<i>Enterococcus columbae</i>	ATCC 51263	<i>Staphylococcus haemolyticus</i>	ATCC 29970
	<i>Enterococcus dispar</i>	ATCC 51266	<i>Staphylococcus hominis</i>	ATCC 27844
	<i>Enterococcus durans</i>	ATCC 19432	<i>Staphylococcus lugdunensis</i>	ATCC 43809
25	<i>Enterococcus faecalis</i>	ATCC 29212	<i>Staphylococcus saprophyticus</i>	ATCC 15305
	<i>Enterococcus faecium</i>	ATCC 19434	<i>Staphylococcus simulans</i>	ATCC 27848
	<i>Enterococcus flavescens</i>	ATCC 49996	<i>Staphylococcus warneri</i>	ATCC 27836
	<i>Enterococcus gallinarum</i>	ATCC 49573	<i>Streptococcus agalactiae</i>	ATCC 13813
	<i>Enterococcus hirae</i>	ATCC 8044	<i>Streptococcus anginosus</i>	ATCC 33397
30	<i>Enterococcus malodoratus</i>	ATCC 43197	<i>Streptococcus bovis</i>	ATCC 33317
	<i>Enterococcus mundtii</i>	ATCC 43186	<i>Streptococcus constellatus</i>	ATCC 27823
	<i>Enterococcus pseudoavium</i>	ATCC 49372	<i>Streptococcus cristatus</i>	ATCC 51100
	<i>Enterococcus raffinosus</i>	ATCC 49427	<i>Streptococcus intermedius</i>	ATCC 27335
	<i>Enterococcus saccharolyticus</i>	ATCC 43076	<i>Streptococcus mitis</i>	ATCC 49456
35	<i>Enterococcus solitarius</i>	ATCC 49428	<i>Streptococcus mitis</i>	ATCC 3639
	<i>Enterococcus sulfureus</i>	ATCC 49903	<i>Streptococcus mutans</i>	ATCC 27175
	<i>Eubacterium lentum</i>	ATCC 49903	<i>Streptococcus parasanguinis</i>	ATCC 15912
	<i>Gemella haemolysans</i>	ATCC 10379	<i>Streptococcus pneumoniae</i>	ATCC 27736
	<i>Gemella morbillorum</i>	ATCC 27842	<i>Streptococcus pneumoniae</i>	ATCC 6303
40	<i>Lactobacillus acidophilus</i>	ATCC 4356	<i>Streptococcus pyogenes</i>	ATCC 19615
	<i>Leuconostoc mesenteroides</i>	ATCC 19225	<i>Streptococcus salivarius</i>	ATCC 7073
	<i>Listeria grayi</i>	ATCC 19120	<i>Streptococcus sanguinis</i>	ATCC 10556
	<i>Listeria grayi</i>	ATCC 19123	<i>Streptococcus suis</i>	ATCC 43765

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Table 10. Bacterial species used to test the specificity of the *Enterococcus*-specific amplification primers derived from *tuf* sequences (continued).

	Strain	Reference number	Strain	Reference number
5	Gram-negative species (n=39)			
	<i>Acidominococcus fermentans</i>	ATCC 2508	<i>Hafnia alvei</i>	ATCC 13337
	<i>Acinetobacter baumannii</i>	ATCC 19606	<i>Klebsiella oxytoca</i>	ATCC 13182
	<i>Alcaligenes faecalis</i>	ATCC 8750	<i>Meganomonas hypermegas</i>	ATCC 25560
	<i>Anaerobiospirillum</i>	ATCC 29305	<i>Mitsukoella multiacidus</i>	ATCC 27723
10	<i>succiniproducens</i>		<i>Moraxella catarrhalis</i>	ATCC 43628
	<i>Anaerorhabdus furcosus</i>	ATCC 25662	<i>Morganella morganii</i>	ATCC 25830
	<i>Bacteroides distasonis</i>	ATCC 8503	<i>Neisseria meningitidis</i>	ATCC 13077
	<i>Bacteroides thetaiotaomicron</i>	ATCC 29741	<i>Pasteurella aerogenes</i>	ATCC 27883
	<i>Bacteroides vulgatus</i>	ATCC 8482	<i>Proteus vulgaris</i>	ATCC 13315
15	<i>Bordetella pertussis</i>	LSPQ 3702	<i>Providencia alcalifaciens</i>	ATCC 9886
	<i>Bulkholderia cepacia</i>	LSPQ 2217	<i>Providencia rettgeri</i>	ATCC 9250
	<i>Butyivibrio fibrinosolvens</i>	ATCC 19171	<i>Pseudomonas aeruginosa</i>	ATCC 27853
	<i>Cardiobacterium hominis</i>	ATCC 15826	<i>Salmonella typhimurium</i>	ATCC 14028
	<i>Citrobacter freundii</i>	ATCC 8090	<i>Serratia marcescens</i>	ATCC 13880
20	<i>Desulfovibrio vulgaris</i>	ATCC 29579	<i>Shigella flexneri</i>	ATCC 12022
	<i>Edwardsiella tarda</i>	ATCC 15947	<i>Shigella sonnei</i>	ATCC 29930
	<i>Enterobacter cloacae</i>	ATCC 13047	<i>Succinivibrio dextrinosolvens</i>	ATCC 19716
	<i>Escherichia coli</i>	ATCC 25922	<i>Tissierella praeacuta</i>	ATCC 25539
	<i>Fusobacterium russii</i>	ATCC 25533	<i>Veillonella parvula</i>	ATCC 10790
25	<i>Haemophilus influenzae</i>	ATCC 9007	<i>Yersinia enterocolitica</i>	ATCC 9610

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases.

	Species	Strain	Accession number	Coding gene*
<u>tuf sequences</u>				
Bacteria				
5	<i>Actinobacillus actinomycetemcomitans</i>	HK1651	Genome project ²	<i>tuf</i>
	<i>Actinobacillus actinomycetemcomitans</i>	HK1651	Genome project ²	<i>tuf</i> (EF-G)
	<i>Agrobacterium tumefaciens</i>		X99673	<i>tuf</i>
	<i>Agrobacterium tumefaciens</i>		X99673	<i>tuf</i> (EF-G)
	<i>Agrobacterium tumefaciens</i>		X99674	<i>tuf</i>
10	<i>Anacystis nidulans</i>	PCC 6301	X17442	<i>tuf</i>
	<i>Aquifex aeolicus</i>	VF5	AE000669	<i>tuf</i>
	<i>Aquifex aeolicus</i>	VF5	AE000669	<i>tuf</i> (EF-G)
	<i>Aquifex pyrophilus</i>		Genome project ²	<i>tuf</i> (EF-G)
	<i>Aquifex pyrophilus</i>		Y15787	<i>tuf</i>
15	<i>Bacillus anthracis</i>	Ames	Genome project ²	<i>tuf</i>
	<i>Bacillus anthracis</i>	Ames	Genome project ²	<i>tuf</i> (EF-G)
	<i>Bacillus halodurans</i>	C-125	AB017508	<i>tuf</i>
	<i>Bacillus halodurans</i>	C-125	AB017508	<i>tuf</i> (EF-G)
	<i>Bacillus stearothermophilus</i>	CCM 2184	AJ000260	<i>tuf</i>
20	<i>Bacillus subtilis</i>	168	D64127	<i>tuf</i>
	<i>Bacillus subtilis</i>	168	D64127	<i>tuf</i> (EF-G)
	<i>Bacillus subtilis</i>	DSM 10	Z99104	<i>tuf</i>
	<i>Bacillus subtilis</i>	DSM 10	Z99104	<i>tuf</i> (EF-G)
	<i>Bacteroides forsythus</i>	ATCC 43037	AB035466	<i>tuf</i>
25	<i>Bacteroides fragilis</i>	DSM 1151	- ¹	<i>tuf</i>
	<i>Bordetella bronchiseptica</i>	RB50	Genome project ²	<i>tuf</i>
	<i>Bordetella pertussis</i>	Tohama 1	Genome project ²	<i>tuf</i>
	<i>Bordetella pertussis</i>	Tohama 1	Genome project ²	<i>tuf</i> (EF-G)
	<i>Borrelia burgdorferi</i>	B31	U78193	<i>tuf</i>
30	<i>Borrelia burgdorferi</i>		AE001155	<i>tuf</i> (EF-G)
	<i>Brevibacterium linens</i>	DSM 20425	X76863	<i>tuf</i>
	<i>Buchnera aphidicola</i>	Ap	Y12307	<i>tuf</i>
	<i>Burkholderia pseudomallei</i>	K96243	Genome project ²	<i>tuf</i> (EF-G)
	<i>Campylobacter jejuni</i>	NCTC 11168	Y17167	<i>tuf</i>
35	<i>Campylobacter jejuni</i>	NCTC 11168	CJ11168X2	<i>tuf</i> (EF-G)
	<i>Chlamydia pneumoniae</i>	CWL029	AE001592	<i>tuf</i>
	<i>Chlamydia pneumoniae</i>	CWL029	AE001639	<i>tuf</i> (EF-G)
	<i>Chlamydia trachomatis</i>		M74221	<i>tuf</i>
	<i>Chlamydia trachomatis</i>	D/UW-3/CX	AE001317	<i>tuf</i> (EF-G)
40	<i>Chlamydia trachomatis</i>	D/UW-3/CX	AE001305	<i>tuf</i>
	<i>Chlamydia trachomatis</i>	F/IC-Cal-13	L22216	<i>tuf</i>
	<i>Chlorobium vibrioforme</i>	DSM 263	X77033	<i>tuf</i>
	<i>Chloroflexus aurantiacus</i>	DSM 636	X76865	<i>tuf</i>
	<i>Clostridium acetobutylicum</i>	ATCC 824	Genome project ²	<i>tuf</i>
45	<i>Clostridium difficile</i>	630	Genome project ²	<i>tuf</i>
	<i>Clostridium difficile</i>	630	Genome project ²	<i>tuf</i> (EF-G)
	<i>Corynebacterium diphtheriae</i>	NCTC 13129	Genome project ²	<i>tuf</i>
	<i>Corynebacterium diphtheriae</i>	NCTC 13129	Genome project ²	<i>tuf</i> (EF-G)
	<i>Corynebacterium glutamicum</i>	ASO 19	X77034	<i>tuf</i>
50	<i>Corynebacterium glutamicum</i>	MJ-233	E09634	<i>tuf</i>
	<i>Coxiella burnetii</i>	Nine Mile phase I	AF136604	<i>tuf</i>
	<i>Cytophaga lytica</i>	DSM 2039	X77035	<i>tuf</i>
	<i>Deinococcus radiodurans</i>	R1	AE001891	<i>tuf</i> (EF-G)
55	<i>Deinococcus radiodurans</i>	R1	AE180092	<i>tuf</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Deinococcus radiodurans</i>	R1	AE002041	<i>tuf</i>
	<i>Deinonema</i> sp.		- ¹	<i>tuf</i>
	<i>Eikenella corrodens</i>	ATCC 23834	Z12610	<i>tuf</i>
	<i>Eikenella corrodens</i>	ATCC 23834	Z12610	<i>tuf</i> (EF-G)
5	<i>Enterococcus faecalis</i>		Genome project ²	<i>tuf</i> (EF-G)
	<i>Escherichia coli</i>		J01690	<i>tuf</i>
	<i>Escherichia coli</i>		J01717	<i>tuf</i>
	<i>Escherichia coli</i>		X00415	<i>tuf</i> (EF-G)
	<i>Escherichia coli</i>		X57091	<i>tuf</i>
10	<i>Escherichia coli</i>	K-12 MG1655	U00006	<i>tuf</i>
	<i>Escherichia coli</i>	K-12 MG1655	U00096	<i>tuf</i>
	<i>Escherichia coli</i>	K-12 MG1655	AE000410	<i>tuf</i> (EF-G)
	<i>Fervidobacterium islandicum</i>	DSM 5733	Y15788	<i>tuf</i>
	<i>Fibrobacter succinogenes</i>	S85	X76866	<i>tuf</i>
15	<i>Flavobacterium ferrugineum</i>	DSM 13524	X76867	<i>tuf</i>
	<i>Flexistipes sinusarabici</i>		X59461	<i>tuf</i>
	<i>Gloeobacter violaceus</i>	PCC 7421	U09433	<i>tuf</i>
	<i>Gloeotheca</i> sp.	PCC 6501	U09434	<i>tuf</i>
	<i>Haemophilus actinomycetemcomitans</i>	HK1651	Genome project ²	<i>tuf</i>
20	<i>Haemophilus ducreyi</i>	35000	AF087414	<i>tuf</i> (EF-G)
	<i>Haemophilus influenzae</i>	Rd	U32739	<i>tuf</i>
	<i>Haemophilus influenzae</i>	Rd	U32746	<i>tuf</i>
	<i>Haemophilus influenzae</i>	Rd	U32739	<i>tuf</i> (EF-G)
	<i>Helicobacter pylori</i>	26695	AE000511	<i>tuf</i>
25	<i>Helicobacter pylori</i>	J99	AE001539	<i>tuf</i> (EF-G)
	<i>Helicobacter pylori</i>	J99	AE001541	<i>tuf</i>
	<i>Herpetosiphon aurantiacus</i>	Hpga1	X76868	<i>tuf</i>
	<i>Klebsiella pneumoniae</i>	M6H 78578	Genome project ²	<i>tuf</i>
	<i>Klebsiella pneumoniae</i>	M6H 78578	Genome project ²	<i>tuf</i> (EF-G)
30	<i>Lactobacillus paracasei</i>		E13922	<i>tuf</i>
	<i>Legionella pneumophila</i>	Philadelphia-1	Genome project ²	<i>tuf</i>
	<i>Leptospira interrogans</i>		AF115283	<i>tuf</i>
	<i>Leptospira interrogans</i>		AF115283	<i>tuf</i> (EF-G)
	<i>Micrococcus luteus</i>	IFO 3333	M17788	<i>tuf</i> (EF-G)
35	<i>Micrococcus luteus</i>	IFO 3333	M17788	<i>tuf</i>
	<i>Moraxella</i> sp.	TAC II 25	AJ249258	<i>tuf</i>
	<i>Mycobacterium avium</i>	104	Genome project ²	<i>tuf</i>
	<i>Mycobacterium avium</i>	104	Genome project ²	<i>tuf</i> (EF-G)
	<i>Mycobacterium bovis</i>	AF2122/97	Genome project ²	<i>tuf</i>
40	<i>Mycobacterium bovis</i>	AF2122/97	Genome project ²	<i>tuf</i> (EF-G)
	<i>Mycobacterium leprae</i>		L13276	<i>tuf</i>
	<i>Mycobacterium leprae</i>		Z14314	<i>tuf</i>
	<i>Mycobacterium leprae</i>		Z14314	<i>tuf</i> (EF-G)
	<i>Mycobacterium leprae</i>	Thai 53	D13869	<i>tuf</i>
45	<i>Mycobacterium tuberculosis</i>	Erdmann	S40925	<i>tuf</i>
	<i>Mycobacterium tuberculosis</i>	H37Rv	AL021943	<i>tuf</i> (EF-G)
	<i>Mycobacterium tuberculosis</i>	H37Rv	Z84395	<i>tuf</i>
	<i>Mycobacterium tuberculosis</i>	y42	AD000005	<i>tuf</i>
	<i>Mycobacterium tuberculosis</i>	CSU#93	Genome project ²	<i>tuf</i>
50	<i>Mycobacterium tuberculosis</i>	CSU#93	Genome project ²	<i>tuf</i> (EF-G)
	<i>Mycoplasma capricolum</i>	PG-31	X16462	<i>tuf</i>
	<i>Mycoplasma genitalium</i>	G37	U39732	<i>tuf</i>
	<i>Mycoplasma genitalium</i>	G37	U39689	<i>tuf</i> (EF-G)
	<i>Mycoplasma hominis</i>		X57136	<i>tuf</i>
55	<i>Mycoplasma hominis</i>	PG21	M57675	<i>tuf</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Mycoplasma pneumoniae</i>	M129	AE000019	<i>tuf</i>
	<i>Mycoplasma pneumoniae</i>	M129	AE000058	<i>tuf</i> (EF-G)
	<i>Neisseria gonorrhoeae</i>	MS11	L36380	<i>tuf</i>
	<i>Neisseria gonorrhoeae</i>	MS11	L36380	<i>tuf</i> (EF-G)
5	<i>Neisseria meningitidis</i>	Z2491	Genome project ²	<i>tuf</i> (EF-G)
	<i>Neisseria meningitidis</i>	Z2491	Genome project ²	<i>tuf</i>
	<i>Pasteurella multocida</i>	Pm70	Genome project ²	<i>tuf</i>
	<i>Peptococcus niger</i>	DSM 20745	X76869	<i>tuf</i>
	<i>Phormidium ectocarp</i>	PCC 7375	U09443	<i>tuf</i>
10	<i>Planobispora rosea</i>	ATCC 53773	U67308	<i>tuf</i>
	<i>Planobispora rosea</i>	ATCC 53733	X98830	<i>tuf</i>
	<i>Planobispora rosea</i>	ATCC 53733	X98830	<i>tuf</i> (EF-G)
	<i>Plectonema boryanum</i>	PCC 73110	U09444	<i>tuf</i>
	<i>Porphyromonas gingivalis</i>	W83	Genome project ²	<i>tuf</i>
15	<i>Porphyromonas gingivalis</i>	W83	Genome project ²	<i>tuf</i> (EF-G)
	<i>Porphyromonas gingivalis</i>	FDC 381	AB035461	<i>tuf</i>
	<i>Porphyromonas gingivalis</i>	W83	AB035462	<i>tuf</i>
	<i>Porphyromonas gingivalis</i>	SUNY 1021	AB035463	<i>tuf</i>
	<i>Porphyromonas gingivalis</i>	A7A1-28	AB035464	<i>tuf</i>
20	<i>Porphyromonas gingivalis</i>	ATCC 33277	AB035465	<i>tuf</i>
	<i>Porphyromonas gingivalis</i>	ATCC 33277	AB035471	<i>tuf</i> (EF-G)
	<i>Prochlorothrix hollandica</i>		U09445	<i>tuf</i>
	<i>Pseudomonas aeruginosa</i>	PAO-1	Genome project ²	<i>tuf</i>
	<i>Pseudomonas putida</i>		Genome project ²	<i>tuf</i>
25	<i>Rickettsia prowazekii</i>	Madrid E	AJ235272	<i>tuf</i>
	<i>Rickettsia prowazekii</i>	Madrid E	AJ235270	<i>tuf</i> (EF-G)
	<i>Rickettsia prowazekii</i>	Madrid E	Z54171	<i>tuf</i> (EF-G)
	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium		X64591	<i>tuf</i> (EF-G)
30	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	LT2 trpE91	X55116	<i>tuf</i>
	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	LT2 trpE91	X55117	<i>tuf</i>
	<i>Serpulina hyodysenteriae</i>	B204	U51635	<i>tuf</i>
35	<i>Serratia marcescens</i>		AF058451	<i>tuf</i>
	<i>Shewanella putrefaciens</i>	DSM 50426	- ¹	<i>tuf</i>
	<i>Shewanella putrefaciens</i>	MR-1	Genome project ²	<i>tuf</i>
	<i>Spirochaeta aurantia</i>	DSM 1902	X76874	<i>tuf</i>
	<i>Staphylococcus aureus</i>		AJ237696	<i>tuf</i> (EF-G)
40	<i>Staphylococcus aureus</i>	EMRSA-16	Genome project ²	<i>tuf</i>
	<i>Staphylococcus aureus</i>	NCTC 8325	Genome project ²	<i>tuf</i>
	<i>Staphylococcus aureus</i>	COL	Genome project ²	<i>tuf</i>
	<i>Staphylococcus aureus</i>	EMRSA-16	Genome project ²	<i>tuf</i> (EF-G)
	<i>Stigmatella aurantiaca</i>	DW4	X82820	<i>tuf</i>
45	<i>Stigmatella aurantiaca</i>	Sg a1	X76870	<i>tuf</i>
	<i>Streptococcus mutans</i>	GS-5 Kuramitsu	U75481	<i>tuf</i>
	<i>Streptococcus mutans</i>	UAB159	Genome project ²	<i>tuf</i>
	<i>Streptococcus oralis</i>	NTCC 11427	P331701	<i>tuf</i>
	<i>Streptococcus pyogenes</i>		Genome project ²	<i>tuf</i> (EF-G)
50	<i>Streptococcus pyogenes</i>	M1-GAS	Genome project ²	<i>tuf</i>
	<i>Streptomyces aureofaciens</i>	ATCC 10762	AF007125	<i>tuf</i>
	<i>Streptomyces cinnamomeus</i>	Tue89	X98831	<i>tuf</i>
	<i>Streptomyces coelicolor</i>	A3(2)	AL031013	<i>tuf</i> (EF-G)
	<i>Streptomyces coelicolor</i>	A3(2)	X77039	<i>tuf</i> (EF-G)
55	<i>Streptomyces coelicolor</i>	M145	X77039	<i>tuf</i>

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Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Streptomyces collinus</i>	BSM 40733	S79408	<i>tuf</i>
	<i>Streptomyces netropsis</i>	Tu1063	AF153618	<i>tuf</i>
	<i>Streptomyces ramocissimus</i>		X67057	<i>tuf</i>
	<i>Streptomyces ramocissimus</i>		X67058	<i>tuf</i>
5	<i>Streptomyces ramocissimus</i>		X67057	<i>tuf</i> (EF-G)
	<i>Synechococcus</i> sp.	PCC 6301	X17442	<i>tuf</i> (EF-G)
	<i>Synechococcus</i> sp.	PCC 6301	X17442	<i>tuf</i>
	<i>Synechocystis</i> sp.	PCC 6803	D90913	<i>tuf</i> (EF-G)
	<i>Synechocystis</i> sp.	PCC 6803	D90913	<i>tuf</i>
10	<i>Synechocystis</i> sp.	PCC 6803	X65159	<i>tuf</i> (EF-G)
	<i>Taxeobacter ocealus</i>	Myx 2105	X77036	<i>tuf</i>
	<i>Thermotoga maritima</i>		Genome project ²	<i>tuf</i> (EF-G)
	<i>Thermotoga maritima</i>		M27479	<i>tuf</i>
	<i>Thermus aquaticus</i>	EP 00276	X66322	<i>tuf</i>
15	<i>Thermus thermophilus</i>	HB8	X16278	<i>tuf</i> (EF-G)
	<i>Thermus thermophilus</i>	HB8	X05977	<i>tuf</i>
	<i>Thermus thermophilus</i>	HB8	X06657	<i>tuf</i>
	<i>Thiomonas cuprina</i>	DSM 5495	U78300	<i>tuf</i>
	<i>Thiomonas cuprina</i>	DSM 5495	U78300	<i>tuf</i> (EF-G)
20	<i>Thiomonas cuprina</i>	Hoe5	X76871	<i>tuf</i>
	<i>Treponema denticola</i>		Genome project ²	<i>tuf</i>
	<i>Treponema denticola</i>		Genome project ²	<i>tuf</i> (EF-G)
	<i>Treponema pallidum</i>		AE001202	<i>tuf</i>
	<i>Treponema pallidum</i>		AE001222	<i>tuf</i> (EF-G)
25	<i>Treponema pallidum</i>		AE001248	<i>tuf</i> (EF-G)
	<i>Ureaplasma urealyticum</i>	ATCC 33697	Z34275	<i>tuf</i>
	<i>Ureaplasma urealyticum</i>	serovar 3 biovar 1	AE002151	<i>tuf</i>
	<i>Ureaplasma urealyticum</i>	serovar 3 biovar 1	AE002151	<i>tuf</i> (EF-G)
	<i>Vibrio cholerae</i>	N16961	Genome project ²	<i>tuf</i>
30	<i>Wolinella succinogenes</i>	DSM 1740	X76872	<i>tuf</i>
	<i>Yersinia pestis</i>	CO-92	Genome project ²	<i>tuf</i>
	<i>Yersinia pestis</i>	CO-92	Genome project ²	<i>tuf</i> (EF-G)
35	Archaeobacteria			
	<i>Archaeoglobus fulgidus</i>		Genome project ²	<i>tuf</i> (EF-G)
	<i>Halobacterium marismortui</i>		X16677	<i>tuf</i>
	<i>Methanobacterium thermoautotrophicum</i>	delta H	AE000877	<i>tuf</i>
40	<i>Methanococcus jannaschii</i>	ATCC 43067	U67486	<i>tuf</i>
	<i>Methanococcus vannielii</i>		X05698	<i>tuf</i>
	<i>Pyrococcus abyssi</i>	Orsay	AJ248285	<i>tuf</i>
	<i>Thermoplasma acidophilum</i>	DSM 1728	X53866	<i>tuf</i>
45	Fungi			
	<i>Absidia glauca</i>	CBS 101.48	X54730	<i>tuf</i> (EF-1)
	<i>Arxula adeninivorans</i>	Ls3	Z47379	<i>tuf</i> (EF-1)
50	<i>Aspergillus oryzae</i>	KBN616	AB007770	<i>tuf</i> (EF-1)
	<i>Aureobasidium pullulans</i>	R106	U19723	<i>tuf</i> (EF-1)
	<i>Candida albicans</i>	SC5314	Genome project ²	<i>tuf</i> (M)
	<i>Candida albicans</i>	SC5314	M29934	<i>tuf</i> (EF-1)
	<i>Candida albicans</i>	SC5314	M29935	<i>tuf</i> (EF-1)
55	<i>Cryptococcus neoformans</i>	B3501	U81803	<i>tuf</i> (EF-1)

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Cryptococcus neoformans</i>	M1-106	U81804	<i>tuf</i> (EF-1)
	<i>Eremothecium gossypii</i>	ATCC 10895	X73978	<i>tuf</i> (EF-1)
	<i>Eremothecium gossypii</i>		A29820	<i>tuf</i> (EF-1)
	<i>Fusarium oxysporum</i>	NRRL 26037	AF008498	<i>tuf</i> (EF-1)
5	<i>Histoplasma capsulatum</i>	186AS	U14100	<i>tuf</i> (EF-1)
	<i>Podospora anserina</i>		X74799	<i>tuf</i> (EF-1)
	<i>Podospora curvicolla</i>	VLV	X96614	<i>tuf</i> (EF-1)
	<i>Prototheca wickerhamii</i>	263-11	AJ245645	<i>tuf</i> (EF-1)
	<i>Puccinia graminis</i>	race 32	X73529	<i>tuf</i> (EF-1)
10	<i>Reclinomonas americana</i>	ATCC 50394	AF007261	<i>tuf</i> (M)
	<i>Rhizomucor racemosus</i>	ATCC 1216B	X17475	<i>tuf</i> (EF-1)
	<i>Rhizomucor racemosus</i>	ATCC 1216B	J02605	<i>tuf</i> (EF-1)
	<i>Rhizomucor racemosus</i>	ATCC 1216B	X17476	<i>tuf</i> (EF-1)
	<i>Rhodotorula mucilaginosa</i>		AF016239	<i>tuf</i> (EF-1)
15	<i>Saccharomyces cerevisiae</i>		K00428	<i>tuf</i> (M)
	<i>Saccharomyces cerevisiae</i>		M59369	<i>tuf</i> (EF-G)
	<i>Saccharomyces cerevisiae</i>		X00779	<i>tuf</i> (EF-1)
	<i>Saccharomyces cerevisiae</i>		X01638	<i>tuf</i> (EF-1)
	<i>Saccharomyces cerevisiae</i>		M10992	<i>tuf</i> (EF-1)
20	<i>Saccharomyces cerevisiae</i>	Alpha S288	X78993	<i>tuf</i> (EF-1)
	<i>Saccharomyces cerevisiae</i>		M15666	<i>tuf</i> (EF-1)
	<i>Saccharomyces cerevisiae</i>		Z35987	<i>tuf</i> (EF-1)
	<i>Saccharomyces cerevisiae</i>	S288C (AB972)	U51033	<i>tuf</i> (EF-1)
	<i>Schizophyllum commune</i>	1-40	X94913	<i>tuf</i> (EF-1)
25	<i>Schizosaccharomyces pombe</i>	972h-	AL021816	<i>tuf</i> (EF-1)
	<i>Schizosaccharomyces pombe</i>	972h-	AL021813	<i>tuf</i> (EF-1)
	<i>Schizosaccharomyces pombe</i>	972h-	D82571	<i>tuf</i> (EF-1)
	<i>Schizosaccharomyces pombe</i>		U42189	<i>tuf</i> (EF-1)
	<i>Schizosaccharomyces pombe</i>	PR745	D89112	<i>tuf</i> (EF-1)
30	<i>Sordaria macrospora</i>	OOO	X96615	<i>tuf</i> (EF-1)
	<i>Trichoderma reesei</i>	QM9414	Z23012	<i>tuf</i> (EF-1)
	<i>Yarrowia lipolytica</i>		AF054510	<i>tuf</i> (EF-1)
35	Parasites			
	<i>Blastocystis hominis</i>	HE87-1	D64080	<i>tuf</i> (EF-1)
	<i>Cryptosporidium parvum</i>		U69697	<i>tuf</i> (EF-1)
	<i>Eimeria tenella</i>	LS18	AI755521	<i>tuf</i> (EF-1)
40	<i>Entamoeba histolytica</i>	HM1:IMSS	X83565	<i>tuf</i> (EF-1)
	<i>Entamoeba histolytica</i>	NIH 200	M92073	<i>tuf</i> (EF-1)
	<i>Giardia lamblia</i>		D14342	<i>tuf</i> (EF-1)
	<i>Kentrophoros</i> sp.		AF056101	<i>tuf</i> (EF-1)
	<i>Leishmania amazonensis</i>	IFLA/BR/67/PH8	M92653	<i>tuf</i> (EF-1)
45	<i>Leishmania braziliensis</i>		U72244	<i>tuf</i> (EF-1)
	<i>Onchocerca volvulus</i>		M64333	<i>tuf</i> (EF-1)
	<i>Porphyra purpurea</i>	Avonport	U08844	<i>tuf</i> (EF-1)
	<i>Plasmodium berghei</i>	ANKA	AJ224150	<i>tuf</i> (EF-1)
	<i>Plasmodium falciparum</i>	K1	X60488	<i>tuf</i> (EF-1)
50	<i>Plasmodium knowlesi</i>	line H	AJ224153	<i>tuf</i> (EF-1)
	<i>Toxoplasma gondii</i>	RH	Y11431	<i>tuf</i> (EF-1)
	<i>Trichomonas tenax</i>	ATCC 30207	D78479	<i>tuf</i> (EF-1)
	<i>Trypanosoma brucei</i>	LVH/75/	U10562	<i>tuf</i> (EF-1)
		USAMRU-K/18		
55	<i>Trypanosoma cruzi</i>	Y	L76077	<i>tuf</i> (EF-1)

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
Human and plants				
	<i>Arabidopsis thaliana</i>	Columbia	X89227	<i>tuf</i> (EF-1)
5	<i>Glycine max</i>	Ceresia	X89058	<i>tuf</i> (EF-1)
	<i>Glycine max</i>	Ceresia	Y15107	<i>tuf</i> (EF-1)
	<i>Glycine max</i>	Ceresia	Y15108	<i>tuf</i> (EF-1)
	<i>Glycine max</i>	Maple Arrow	X66062	<i>tuf</i> (EF-1)
	<i>Homo sapiens</i>		X03558	<i>tuf</i> (EF-1)
10	<i>Pyramimonas disomata</i>		AB008010	<i>tuf</i>
<u>atpD sequences</u>				
15	Bacteria			
	<i>Acetobacterium woodii</i>	DSM 1030	U10505	<i>atpD</i>
	<i>Actinobacillus actinomycetemcomitans</i>	HK1651	Genome project ²	<i>atpD</i>
	<i>Bacillus anthracis</i>	Ames	Genome project ²	<i>atpD</i>
20	<i>Bacillus firmus</i>	OF4	M60117	<i>atpD</i>
	<i>Bacillus megaterium</i>	QM B1551	M20255	<i>atpD</i>
	<i>Bacillus stearothermophilus</i>		D38058	<i>atpD</i>
	<i>Bacillus stearothermophilus</i>	IFO1035	D38060	<i>atpD</i>
	<i>Bacillus subtilis</i>	168	Z28592	<i>atpD</i>
25	<i>Bacteroides fragilis</i>	DSM 2151	M22247	<i>atpD</i>
	<i>Bordetella bronchiseptica</i>	RB50	Genome project ²	<i>atpD</i>
	<i>Bordetella pertussis</i>	Tohama 1	Genome project ²	<i>atpD</i>
	<i>Borrelia burgdorferi</i>	B31	AE001122	<i>atpD</i> (V)
	<i>Burkholderia cepacia</i>	DSM50181	X76877	<i>atpD</i>
30	<i>Burkholderia pseudomallei</i>	K96243	Genome project ²	<i>atpD</i>
	<i>Campylobacter jejuni</i>	NCTC 11168	CJ11168X1	<i>atpD</i>
	<i>Chlamydia pneumoniae</i>		Genome project ²	<i>atpD</i> (V)
	<i>Chlamydia trachomatis</i>	MoPn	Genome project ²	<i>atpD</i> (V)
	<i>Chlorobium vibrioforme</i>	DSM 263	X76873	<i>atpD</i>
35	<i>Citrobacter freundii</i>	JEO503	AF037156	<i>atpD</i>
	<i>Clostridium acetobutylicum</i>	ATCC 824	Genome project ²	<i>atpD</i>
	<i>Clostridium acetobutylicum</i>	DSM 792	AF101055	<i>atpD</i>
	<i>Clostridium difficile</i>	630	Genome project ²	<i>atpD</i>
	<i>Corynebacterium diphtheriae</i>	NCTC13129	Genome project ²	<i>atpD</i>
40	<i>Corynebacterium glutamicum</i>	ASO 19	X76875	<i>atpD</i>
	<i>Corynebacterium glutamicum</i>	MJ-233	E09634	<i>atpD</i>
	<i>Cytophaga lytica</i>	DSM 2039	M22535	<i>atpD</i>
	<i>Enterobacter aerogenes</i>	DSM 30053	- ³	<i>atpD</i>
	<i>Enterococcus faecalis</i>	V583	Genome project ²	<i>atpD</i> (V)
45	<i>Enterococcus hirae</i>		M90060	<i>atpD</i>
	<i>Enterococcus hirae</i>	ATCC 9790	D17462	<i>atpD</i> (V)
	<i>Escherichia coli</i>		J01594	<i>atpD</i>
	<i>Escherichia coli</i>		M25464	<i>atpD</i>
	<i>Escherichia coli</i>		V00267	<i>atpD</i>
50	<i>Escherichia coli</i>		V00311	<i>atpD</i>
	<i>Escherichia coli</i>	K12 MG1655	L10328	<i>atpD</i>
	<i>Flavobacterium ferrugineum</i>	DSM 13524	- ³	<i>atpD</i>
	<i>Haemophilus actinomycetemcomitans</i>		Genome project ²	<i>atpD</i>
	<i>Haemophilus influenzae</i>	Rd	U32730	<i>atpD</i>
55	<i>Helicobacter pylori</i>	NCTC 11638	AF004014	<i>atpD</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Helicobacter pylori</i>	26695	Genome project ²	<i>atpD</i>
	<i>Helicobacter pylori</i>	J99	Genome project ²	<i>atpD</i>
	<i>Klebsiella pneumoniae</i>	M6H 78578	Genome project ²	<i>atpD</i>
	<i>Lactobacillus casei</i>	DSM 20021	X64542	<i>atpD</i>
5	<i>Legionella pneumophila</i>	Philadelphia-1	Genome project ²	<i>atpD</i>
	<i>Moorella thermoacetica</i>	ATCC 39073	U64318	<i>atpD</i>
	<i>Mycobacterium avium</i>	104	Genome project ²	<i>atpD</i>
	<i>Mycobacterium bovis</i>	AF2122/97	Genome project ²	<i>atpD</i>
	<i>Mycobacterium leprae</i>		U15186	<i>atpD</i>
10	<i>Mycobacterium leprae</i>		Genome project ²	<i>atpD</i>
	<i>Mycobacterium tuberculosis</i>	H37Rv	Z73419	<i>atpD</i>
	<i>Mycobacterium tuberculosis</i>	CSU#93	Genome project ²	<i>atpD</i>
	<i>Mycoplasma gallisepticum</i>		X64256	<i>atpD</i>
	<i>Mycoplasma genitalium</i>	G37	U39725	<i>atpD</i>
15	<i>Mycoplasma pneumoniae</i>	M129	U43738	<i>atpD</i>
	<i>Neisseria gonorrhoeae</i>	FA 1090	Genome project ²	<i>atpD</i>
	<i>Neisseria meningitidis</i>	Z2491	Genome project ²	<i>atpD</i>
	<i>Pasteurella multocida</i>	Pm70	Genome project ²	<i>atpD</i>
	<i>Pectinatus frisingensis</i>	DSM 20465	X64543	<i>atpD</i>
20	<i>Peptococcus niger</i>	DSM 20475	X76878	<i>atpD</i>
	<i>Pirellula marina</i>	IFAM 1313	X57204	<i>atpD</i>
	<i>Porphyromonas gingivalis</i>	W83	Genome project ²	<i>atpD</i> (V)
	<i>Propionigenium modestum</i>	DSM 2376	X58461	<i>atpD</i>
	<i>Pseudomonas aeruginosa</i>	PAO1	Genome project ²	<i>atpD</i>
25	<i>Pseudomonas putida</i>		Genome project ²	<i>atpD</i>
	<i>Rhodobacter capsulatus</i>	B100	X99599	<i>atpD</i>
	<i>Rhodospirillum rubrum</i>		X02499	<i>atpD</i>
	<i>Rickettsia prowazekii</i>	F-12	AF036246	<i>atpD</i>
	<i>Rickettsia prowazekii</i>	Madrid	Genome project ²	<i>atpD</i>
30	<i>Ruminococcus albus</i>	7ATCC	AB006151	<i>atpD</i>
	<i>Salmonella bongori</i>	JEO4162	AF037155	<i>atpD</i>
	<i>Salmonella bongori</i>	BR1859	AF037154	<i>atpD</i>
	<i>Salmonella choleraesuis</i>	S83769	AF037146	<i>atpD</i>
	subsp. <i>arizonae</i>			
35	<i>Salmonella choleraesuis</i>	u24	AF037147	<i>atpD</i>
	subsp. <i>arizonae</i>			
	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Dublin	K228	AF037140	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Dublin	K771	AF037139	<i>atpD</i>
40	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Infantis	Div36-86	AF037142	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Tennessee	Div95-86	AF037143	<i>atpD</i>
45	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	LT2	AF037141	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	DS210/89	AF037149	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	JEO307	AF037148	<i>atpD</i>
50	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	S109671	AF037150	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>diarizonae</i>	S84366	AF037151	<i>atpD</i>
	<i>Salmonella choleraesuis</i> subsp. <i>houtenae</i>			
55	<i>Salmonella choleraesuis</i>	S84098	AF037152	<i>atpD</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	subsp. <i>houtenae</i>			
	<i>Salmonella choleraesuis</i>	BR2047	AF037153	<i>atpD</i>
	subsp. <i>indica</i>			
	<i>Salmonella choleraesuis</i>	NSC72	AF037144	<i>atpD</i>
5	subsp. <i>salamae</i>			
	<i>Salmonella choleraesuis</i>	S114655	AF037145	<i>atpD</i>
	subsp. <i>salamae</i>			
	<i>Shewanella putrefaciens</i>	MR-1	Genome project ²	<i>atpD</i>
	<i>Staphylococcus aureus</i>	COL	Genome project ²	<i>atpD</i>
10	<i>Stigmatella aurantiaca</i>	Sga1	X76879	<i>atpD</i>
	<i>Streptococcus bovis</i>	JB-1	AB009314	<i>atpD</i>
	<i>Streptococcus mutans</i>	GS-5	U31170	<i>atpD</i>
	<i>Streptococcus mutans</i>	UAB159	Genome project ²	<i>atpD</i>
	<i>Streptococcus pneumoniae</i>	Type 4	Genome project ²	<i>atpD</i> (V)
15	<i>Streptococcus pneumoniae</i>	Type 4	Genome project ²	<i>atpD</i>
	<i>Streptococcus pyogenes</i>	M1-GAS	Genome project ²	<i>atpD</i> (V)
	<i>Streptococcus pyogenes</i>	M1-GAS	Genome project ²	<i>atpD</i>
	<i>Streptococcus sanguinis</i>	10904	AF001955	<i>atpD</i>
	<i>Streptomyces lividans</i>	1326	Z22606	<i>atpD</i>
20	<i>Thermus thermophilus</i>	HB8	D63799	<i>atpD</i> (V)
	<i>Thiobacillus ferrooxidans</i>	ATCC 33020	M81087	<i>atpD</i>
	<i>Treponema pallidum</i>	Nichols	AE001228	<i>atpD</i> (V)
	<i>Vibrio alginolyticus</i>		X16050	<i>atpD</i>
	<i>Vibrio cholerae</i>	N16961	Genome project ²	<i>atpD</i>
25	<i>Wolinella succinogenes</i>	DSM 1470	X76880	<i>atpD</i>
	<i>Yersinia enterocolitica</i>	NCTC 10460	AF037157	<i>atpD</i>
	<i>Yersinia pestis</i>	CO-92	Genome project ²	<i>atpD</i>
30	Archaeobacteria			
	<i>Archaeoglobus fulgidus</i>	DSM 4304	AE001023	<i>atpD</i> (V)
	<i>Halobacterium salinarum</i>		S56356	<i>atpD</i> (V)
	<i>Haloferax volcanii</i>	WR 340	X79516	<i>atpD</i>
35	<i>Methanococcus jannaschii</i>	DSM 2661	U67477	<i>atpD</i> (V)
	<i>Methanosarcina barkeri</i>	DSM 800	J04836	<i>atpD</i> (V)
	Fungi			
40	<i>Candida albicans</i>	SC5314	Genome project ²	<i>atpD</i>
	<i>Candida tropicalis</i>		M64984	<i>atpD</i> (V)
	<i>Kluyveromyces lactis</i>	2359/152	U37764	<i>atpD</i>
	<i>Neurospora crassa</i>		X53720	<i>atpD</i>
45	<i>Saccharomyces cerevisiae</i>		M12082	<i>atpD</i>
	<i>Saccharomyces cerevisiae</i>	X2180-1A	J05409	<i>atpD</i> (V)
	<i>Schizosaccharomyces pombe</i>	972 h-	S47814	<i>atpD</i> (V)
	<i>Schizosaccharomyces pombe</i>	972 h-	M57956	<i>atpD</i>
50	Parasites			
	<i>Giardia lamblia</i>	WB	U18938	<i>atpD</i>
	<i>Plasmodium falciparum</i>	3D7	L08200	<i>atpD</i> (V)
55	<i>Trypanosoma congolense</i>	IL3000	Z25814	<i>atpD</i> (V)

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
Human and plants				
5	<i>Homo sapiens</i>		L09234	<i>atpD</i> (V)
	<i>Homo sapiens</i>		M27132	<i>atpD</i>
<u>recA sequences</u>				
10	Bacteria			
	<i>Acetobacter aceti</i>	no. 1023	S60630	<i>recA</i>
	<i>Acetobacter altoacetigenes</i>	MH-24	E05290	<i>recA</i>
15	<i>Acetobacter polyoxogenes</i>	NBI 1028	D13183	<i>recA</i>
	<i>Acholeplasma laidlawii</i>	8195	M81465	<i>recA</i>
	<i>Acidiphilium facilis</i>	ATCC 35904	D16538	<i>recA</i>
	<i>Acidothermus cellulolyticus</i>	ATCC 43068	AJ006705	<i>recA</i>
	<i>Acinetobacter calcoaceticus</i>	BD413/ADP1	L26100	<i>recA</i>
20	<i>Actinobacillus actinomycetemcomitans</i>	HK1651	Genome project ²	<i>recA</i>
	<i>Aeromonas salmonicida</i>	A449	U83688	<i>recA</i>
	<i>Agrobacterium tumefaciens</i>	C58	L07902	<i>recA</i>
	<i>Allochrochromatium vinosum</i>		AJ000677	<i>recA</i>
	<i>Aquifex aeolicus</i>	VF5	AE000775	<i>recA</i>
25	<i>Aquifex pyrophilus</i>	Kol5a	L23135	<i>recA</i>
	<i>Azotobacter vinelandii</i>		S96898	<i>recA</i>
	<i>Bacillus stearothermophilus</i>	10	Genome project ²	<i>recA</i>
	<i>Bacillus subtilis</i>	PB1831	U87792	<i>recA</i>
	<i>Bacillus subtilis</i>	168	Z99112	<i>recA</i>
30	<i>Bacteroides fragilis</i>		M63029	<i>recA</i>
	<i>Bifidobacterium breve</i>	NCFB 2258	AF094756	<i>recA</i>
	<i>Blastochloris viridis</i>	DSM 133	AF022175	<i>recA</i>
	<i>Bordetella pertussis</i>	165	X53457	<i>recA</i>
	<i>Bordetella pertussis</i>	Tohama I	Genome project ²	<i>recA</i>
35	<i>Borrelia burgdorferi</i>	Sh-2-82	U23457	<i>recA</i>
	<i>Borrelia burgdorferi</i>	B31	AE001124	<i>recA</i>
	<i>Brevibacterium flavum</i>	MJ-233	E10390	<i>recA</i>
	<i>Brucella abortus</i>	2308	L00679	<i>recA</i>
	<i>Burkholderia cepacia</i>	ATCC 17616	U70431	<i>recA</i>
40	<i>Burkholderia cepacia</i>		D90120	<i>recA</i>
	<i>Burkholderia pseudomallei</i>	K96243	Genome project ²	<i>recA</i>
	<i>Campylobacter fetus</i> subsp. <i>fetus</i>	23D	AF020677	<i>recA</i>
	<i>Campylobacter jejuni</i>	81-176	U03121	<i>recA</i>
	<i>Campylobacter jejuni</i>	NCTC 11168	AL139079	<i>recA</i>
45	<i>Chlamydia trachomatis</i>	L2	U16739	<i>recA</i>
	<i>Chlamydia trachomatis</i>	D/UW-3/CX	AE001335	<i>recA</i>
	<i>Chlamydoghila pneumoniae</i>	CWL029	AE001658	<i>recA</i>
	<i>Chloroflexus aurantiacus</i>	J-10-fl	AF037259	<i>recA</i>
	<i>Clostridium acetobutylicum</i>		M94057	<i>recA</i>
50	<i>Clostridium perfringens</i>	13	U61497	<i>recA</i>
	<i>Corynebacterium diphtheriae</i>	NCTC13129	Genome project ²	<i>recA</i>
	<i>Corynebacterium glutamicum</i>	AS019	U14965	<i>recA</i>
	<i>Corynebacterium pseudotuberculosis</i>	C231	U30387	<i>recA</i>
	<i>Deinococcus radiodurans</i>	KD8301	AB005471	<i>recA</i>
55	<i>Deinococcus radiodurans</i>	R1	U01876	<i>recA</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Enterobacter agglomerans</i>	339	L03291	<i>recA</i>
	<i>Enterococcus faecalis</i>	OGIX	M81466	<i>recA</i>
	<i>Erwinia carotovora</i>		X55554	<i>recA</i>
	<i>Escherichia coli</i>		J01672	<i>recA</i>
5	<i>Escherichia coli</i>		X55552	<i>recA</i>
	<i>Escherichia coli</i>	K-12	AE000354	<i>recA</i>
	<i>Frankia alni</i>	Arl3	AJ006707	<i>recA</i>
	<i>Gluconobacter oxydans</i>		U21001	<i>recA</i>
	<i>Haemophilus influenzae</i>	Rd	U32687	<i>recA</i>
10	<i>Haemophilus influenzae</i>	Rd	U32741	<i>recA</i>
	<i>Haemophilus influenzae</i>	Rd	L07529	<i>recA</i>
	<i>Helicobacter pylori</i>	69A	Z35478	<i>recA</i>
	<i>Helicobacter pylori</i>	26695	AE000536	<i>recA</i>
	<i>Helicobacter pylori</i>	J99	AE001453	<i>recA</i>
15	<i>Klebsiella pneumoniae</i>	M6H 78578	Genome project ²	<i>recA</i>
	<i>Lactococcus lactis</i>	ML3	M88106	<i>recA</i>
	<i>Legionella pneumophila</i>		X55453	<i>recA</i>
	<i>Leptospira biflexa</i>	serovar patoc	U32625	<i>recA</i>
	<i>Leptospira interrogans</i>	serovar pomona	U29169	<i>recA</i>
20	<i>Magnetospirillum magnetotacticum</i>	MS-1	X17371	<i>recA</i>
	<i>Methylobacillus flagellatus</i>	MFK1	M35325	<i>recA</i>
	<i>Methylomonas clara</i>	ATCC 31226	X59514	<i>recA</i>
	<i>Mycobacterium avium</i>	104	Genome project ²	<i>recA</i>
	<i>Mycobacterium bovis</i>	AF122/97	Genome project ²	<i>recA</i>
25	<i>Mycobacterium leprae</i>		X73822	<i>recA</i>
	<i>Mycobacterium tuberculosis</i>	H37Rv	X58485	<i>recA</i>
	<i>Mycobacterium tuberculosis</i>	CSU#93	Genome project ²	<i>recA</i>
	<i>Mycoplasma genitalium</i>	G37	U39717	<i>recA</i>
	<i>Mycoplasma mycoides</i>	GM9	L22073	<i>recA</i>
30	<i>Mycoplasma pneumoniae</i>	ATCC 29342	MPAE000033	<i>recA</i>
	<i>Mycoplasma pulmonis</i>	KD735	L22074	<i>recA</i>
	<i>Myxococcus xanthus</i>		L40368	<i>recA</i>
	<i>Myxococcus xanthus</i>		L40367	<i>recA</i>
	<i>Neisseria animalis</i>	NCTC 10212	U57910	<i>recA</i>
35	<i>Neisseria cinerea</i>	LCDC 81-176	AJ223869	<i>recA</i>
	<i>Neisseria cinerea</i>	LNP 1646	U57906	<i>recA</i>
	<i>Neisseria cinerea</i>	NCTC 10294	AJ223871	<i>recA</i>
	<i>Neisseria cinerea</i>	Vedros M601	AJ223870	<i>recA</i>
	<i>Neisseria elongata</i>	CCUG 2131	AJ223882	<i>recA</i>
40	<i>Neisseria elongata</i>	CCUG 4165A	AJ223880	<i>recA</i>
	<i>Neisseria elongata</i>	NCTC 10660	AJ223881	<i>recA</i>
	<i>Neisseria elongata</i>	NCTC 11050	AJ223878	<i>recA</i>
	<i>Neisseria elongata</i>	NHITCC 2376	AJ223877	<i>recA</i>
	<i>Neisseria elongata</i>	CCUG 4557	AJ223879	<i>recA</i>
45	subsp. <i>intermedia</i>			
	<i>Neisseria flava</i>	Bangor 9	AJ223873	<i>recA</i>
	<i>Neisseria flavescens</i>	LNP 444	U57907	<i>recA</i>
	<i>Neisseria gonorrhoeae</i>	CH95	U57902	<i>recA</i>
	<i>Neisseria gonorrhoeae</i>	FA19	X64842	<i>recA</i>
50	<i>Neisseria gonorrhoeae</i>	MS11	X17374	<i>recA</i>
	<i>Neisseria gonorrhoeae</i>		Genome project ²	<i>recA</i>
	<i>Neisseria lactamica</i>	CCUG 7757	AJ223866	<i>recA</i>
	<i>Neisseria lactamica</i>	CCUG 7852	Y11819	<i>recA</i>
	<i>Neisseria lactamica</i>	LCDC 77-143	Y11818	<i>recA</i>
55	<i>Neisseria lactamica</i>	LCDC 80-111	AJ223864	<i>recA</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Neisseria lactamica</i>	LCDC 845	AJ223865	<i>recA</i>
	<i>Neisseria lactamica</i>	NCTC 10617	U57905	<i>recA</i>
	<i>Neisseria lactamica</i>	NCTC 10618	AJ223863	<i>recA</i>
	<i>Neisseria meningitidis</i>	44/46	X64849	<i>recA</i>
5	<i>Neisseria meningitidis</i>	Bangor 13	AJ223868	<i>recA</i>
	<i>Neisseria meningitidis</i>	HF116	X64848	<i>recA</i>
	<i>Neisseria meningitidis</i>	HF130	X64844	<i>recA</i>
	<i>Neisseria meningitidis</i>	HF46	X64847	<i>recA</i>
	<i>Neisseria meningitidis</i>	M470	X64850	<i>recA</i>
10	<i>Neisseria meningitidis</i>	N94II	X64846	<i>recA</i>
	<i>Neisseria meningitidis</i>	NCTC 8249	AJ223867	<i>recA</i>
	<i>Neisseria meningitidis</i>	P63	X64845	<i>recA</i>
	<i>Neisseria meningitidis</i>	S3446	U57903	<i>recA</i>
	<i>Neisseria meningitidis</i>	FAM18	Genome project ²	<i>recA</i>
15	<i>Neisseria mucosa</i>	LNP 405	U57908	<i>recA</i>
	<i>Neisseria mucosa</i>	Vedros M1801	AJ223875	<i>recA</i>
	<i>Neisseria perflava</i>	CCUG 17915	AJ223876	<i>recA</i>
	<i>Neisseria perflava</i>	LCDC 85402	AJ223862	<i>recA</i>
	<i>Neisseria pharyngis</i> var. <i>flava</i>	NCTC 4590	U57909	<i>recA</i>
20	<i>Neisseria polysaccharea</i>	CCUG 18031	Y11815	<i>recA</i>
	<i>Neisseria polysaccharea</i>	CCUG 24845	Y11816	<i>recA</i>
	<i>Neisseria polysaccharea</i>	CCUG 24846	Y11814	<i>recA</i>
	<i>Neisseria polysaccharea</i>	INS MA 3008	Y11817	<i>recA</i>
	<i>Neisseria polysaccharea</i>	NCTC 11858	U57904	<i>recA</i>
25	<i>Neisseria sicca</i>	NRL 30016	AJ223872	<i>recA</i>
	<i>Neisseria subflava</i>	NRL 30017	AJ223874	<i>recA</i>
	<i>Paracoccus denitrificans</i>	DSM 413	U59631	<i>recA</i>
	<i>Pasteurella multocida</i>		X99324	<i>recA</i>
	<i>Porphyromonas gingivalis</i>	W83	U70054	<i>recA</i>
30	<i>Prevotella ruminicola</i>	JCM 8958	U61227	<i>recA</i>
	<i>Proteus mirabilis</i>	pG1300	X14870	<i>recA</i>
	<i>Proteus vulgaris</i>		X55555	<i>recA</i>
	<i>Pseudomonas aeruginosa</i>		X05691	<i>recA</i>
	<i>Pseudomonas aeruginosa</i>	PAM 7	X52261	<i>recA</i>
35	<i>Pseudomonas aeruginosa</i>	PAO12	D13090	<i>recA</i>
	<i>Pseudomonas fluorescens</i>	OE 28.3	M96558	<i>recA</i>
	<i>Pseudomonas putida</i>		L12684	<i>recA</i>
	<i>Pseudomonas putida</i>	PpS145	U70864	<i>recA</i>
	<i>Rhizobium leguminosarum</i>	VF39	X59956	<i>recA</i>
40	biovar <i>viciae</i>			
	<i>Rhizobium phaseoli</i>	CNPAF512	X62479	<i>recA</i>
	<i>Rhodobacter capsulatus</i>	J50	X82183	<i>recA</i>
	<i>Rhodobacter sphaeroides</i>	2.4.1	X72705	<i>recA</i>
	<i>Rhodopseudomonas palustris</i>	N 7	D84467	<i>recA</i>
45	<i>Rickettsia prowazekii</i>	Madrid E	AJ235273	<i>recA</i>
	<i>Rickettsia prowazekii</i>	Madrid E	U01959	<i>recA</i>
	<i>Serratia marcescens</i>		M22935	<i>recA</i>
	<i>Shigella flexneri</i>		X55553	<i>recA</i>
	<i>Shigella sonnei</i>	KNIH104S	AF101227	<i>recA</i>
50	<i>Sinorhizobium meliloti</i>	2011	X59957	<i>recA</i>
	<i>Staphylococcus aureus</i>		L25893	<i>recA</i>
	<i>Streptococcus gordonii</i>	Challis V288	L20574	<i>recA</i>
	<i>Streptococcus mutans</i>	UA96	M81468	<i>recA</i>
	<i>Streptococcus mutans</i>	GS-5	M61897	<i>recA</i>
55	<i>Streptococcus pneumoniae</i>		Z17307	<i>recA</i>

Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

	Species	Strain	Accession number	Coding gene*
	<i>Streptococcus pneumoniae</i>	R800	Z34303	<i>recA</i>
	<i>Streptococcus pyogenes</i>	NZ131	U21934	<i>recA</i>
	<i>Streptococcus pyogenes</i>	D471	M81469	<i>recA</i>
	<i>Streptococcus salivarius</i>		M94062	<i>recA</i>
5	subsp. <i>thermophilus</i>			
	<i>Streptomyces ambofaciens</i>	DSM 40697	Z30324	<i>recA</i>
	<i>Streptomyces coelicolor</i>	A3(2)	AL020958	<i>recA</i>
	<i>Streptomyces lividans</i>	TK24	X76076	<i>recA</i>
	<i>Streptomyces rimosus</i>	R6	X94233	<i>recA</i>
10	<i>Streptomyces venezuelae</i>	ATCC10712	U04837	<i>recA</i>
	<i>Synechococcus</i> sp.	PR6	M29495	<i>recA</i>
	<i>Synechocystis</i> sp.	PCC6803	D90917	<i>recA</i>
	<i>Thermotoga maritima</i>		L23425	<i>recA</i>
	<i>Thermotoga maritima</i>		AE001823	<i>recA</i>
15	<i>Thermus aquaticus</i>		L20095	<i>recA</i>
	<i>Thermus thermophilus</i>	HB8	D17392	<i>recA</i>
	<i>Thiobacillus ferrooxidans</i>		M26933	<i>recA</i>
	<i>Treponema denticola</i>		Genome project ²	<i>recA</i>
	<i>Treponema pallidum</i>	Nichols	AE001243	<i>recA</i>
20	<i>Vibrio anguillarum</i>		M80525	<i>recA</i>
	<i>Vibrio cholerae</i>	017	X71969	<i>recA</i>
	<i>Vibrio cholerae</i>	2740-80	U10162	<i>recA</i>
	<i>Vibrio cholerae</i>	569B	L42384	<i>recA</i>
	<i>Vibrio cholerae</i>	M549	AF117881	<i>recA</i>
25	<i>Vibrio cholerae</i>	M553	AF117882	<i>recA</i>
	<i>Vibrio cholerae</i>	M645	AF117883	<i>recA</i>
	<i>Vibrio cholerae</i>	M793	AF117878	<i>recA</i>
	<i>Vibrio cholerae</i>	M794	AF117880	<i>recA</i>
	<i>Vibrio cholerae</i>	M967	AF117879	<i>recA</i>
30	<i>Xanthomonas citri</i>	XW47	AF006590	<i>recA</i>
	<i>Xanthomonas oryzae</i>		AF013600	<i>recA</i>
	<i>Xenorhabdus bovienii</i>	T228/1	U87924	<i>recA</i>
	<i>Xenorhabdus nematophilus</i>	AN6	AF127333	<i>recA</i>
	<i>Yersinia pestis</i>	231	X75336	<i>recA</i>
35	<i>Yersinia pestis</i>	CO-92	Genome project ²	<i>recA</i>
Fungi, parasites, human and plants				
40	<i>Anabaena variabilis</i>	ATCC 29413	M29680	<i>recA</i>
	<i>Arabidopsis thaliana</i>		U43652	<i>recA</i> (Rad51)
	<i>Candida albicans</i>		U39808	<i>recA</i> (Dmc1)
	<i>Coprinus cinereus</i>	Okayama-7	U21905	<i>recA</i> (Rad51)
	<i>Emericella nidulans</i>		Z80341	<i>recA</i> (Rad51)
45	<i>Gallus gallus</i>		L09655	<i>recA</i> (Rad51)
	<i>Homo sapiens</i>		D13804	<i>recA</i> (Rad51)
	<i>Homo sapiens</i>		D63882	<i>recA</i> (Dmc1)
	<i>Leishmania major</i>	Friedlin	AF062379	<i>recA</i> (Rad51)
	<i>Leishmania major</i>	Friedlin	AF062380	<i>recA</i> (Dmc1)
50	<i>Mus musculus</i>		D58419	<i>recA</i> (Dmc1)
	<i>Neurospora crassa</i>	74-OR23-1A	D29638	<i>recA</i> (Rad51)
	<i>Saccharomyces cerevisiae</i>		D10023	<i>recA</i> (Rad51)
	<i>Schizosaccharomyces pombe</i>		Z22691	<i>recA</i> (Rad51)
	<i>Schizosaccharomyces pombe</i>	972h-	AL021817	<i>recA</i> (Dmc1)
55	<i>Tetrahymena thermophila</i>	PB9R	AF064516	<i>recA</i> (Rad51)

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Table 11. Microbial species for which *tuf* and/or *atpD* and/or *recA* sequences are available in public databases (continued).

Species	Strain	Accession number	Coding gene*
<i>Trypanosoma brucei</i>	stock 427	Y13144	<i>recA</i> (Rad51)
<i>Ustilago maydis</i>		U62484	<i>recA</i> (Rad51)
<i>Xenopus laevis</i>		D38488	<i>recA</i> (Rad51)
5 <i>Xenopus laevis</i>		D38489	<i>recA</i> (Rad51)

* *tuf* indicates *tuf* sequences, including *tuf* genes, *fusA* genes and *fusA-tuf* intergenic spacers.

tuf (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu

10 *tuf* (EF-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1 α)

tuf (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin

atpD indicates *atpD* sequences of the F-type

atpD (V) indicates *atpD* sequences of the V-Type

recA indicates *recA* sequences

15 *recA* (Rad51) indicates *rad51* sequences or homologs

recA (Dmc1) indicates *dmc1* sequences or homologs

¹ Nucleotides sequences published in Arch. Microbiol. 1990 153:241-247

² These sequences are from the TIGR database (<http://www.tigr.org/tdb/tdb.html>)

³ Nucleotides sequences published in FEMS Microbiology Letters 1988 50:101-106

Table 12. Bacterial species used to test the specificity of the *Staphylococcus*-specific amplification primers derived from *tuf* sequences.

	Strain	Reference number	Strain	Reference number
5	Staphylococcal species (n=27)		Other Gram-positive bacteria (n=20)	
	<i>Staphylococcus arlettae</i>	ATCC 43957	<i>Bacillus subtilis</i>	ATCC 27370
	<i>Staphylococcus aureus</i>	ATCC 35844	<i>Enterococcus avium</i>	ATCC 14025
	subsp. <i>anaerobius</i>			
10	<i>Staphylococcus aureus</i>	ATCC 43300	<i>Enterococcus durans</i>	ATCC 19432
	subsp. <i>aureus</i>			
	<i>Staphylococcus auricularis</i>	ATCC 33753	<i>Enterococcus faecalis</i>	ATCC 19433
	<i>Staphylococcus capitis</i>	ATCC 27840	<i>Enterococcus faecium</i>	ATCC 19434
	subsp. <i>capitis</i>			
15	<i>Staphylococcus caprae</i>	ATCC 35538	<i>Enterococcus flavescens</i>	ATCC 49996
	<i>Staphylococcus carnosus</i>	ATCC 51365	<i>Enterococcus gallinarum</i>	ATCC 49573
	<i>Staphylococcus chromogenes</i>	ATCC 43764	<i>Lactobacillus acidophilus</i>	ATCC 4356
	<i>Staphylococcus cohnii</i>	DSM 20260	<i>Lactococcus lactis</i>	ATCC 11454
	subsp. <i>urealyticum</i>			
20	<i>Staphylococcus delphini</i>	ATCC 49171	<i>Listeria innocua</i>	ATCC 33090
	<i>Staphylococcus epidermidis</i>	ATCC 14990	<i>Listeria ivanovii</i>	ATCC 19119
	<i>Staphylococcus equorum</i>	ATCC 43958	<i>Listeria monocytogenes</i>	ATCC 15313
	<i>Staphylococcus felis</i>	ATCC 49168	<i>Macrococcus caseolyticus</i>	ATCC 13548
	<i>Staphylococcus gallinarum</i>	ATCC 35539	<i>Streptococcus agalactiae</i>	ATCC 13813
25	<i>Staphylococcus haemolyticus</i>	ATCC 29970	<i>Streptococcus anginosus</i>	ATCC 33397
	<i>Staphylococcus hominis</i>	ATCC 27844	<i>Streptococcus bovis</i>	ATCC 33317
	<i>Staphylococcus hyicus</i>	ATCC 11249	<i>Streptococcus mutans</i>	ATCC 25175
	<i>Staphylococcus intermedius</i>	ATCC 29663	<i>Streptococcus pneumoniae</i>	ATCC 6303
	<i>Staphylococcus kloosis</i>	ATCC 43959	<i>Streptococcus pyogenes</i>	ATCC 19615
30	<i>Staphylococcus lentus</i>	ATCC 29070	<i>Streptococcus salivarius</i>	ATCC 7073
	<i>Staphylococcus lugdunensis</i>	ATCC 43809		
	<i>Staphylococcus saprophyticus</i>	ATCC 15305		
	<i>Staphylococcus schleiferi</i>	ATCC 49545		
	subsp. <i>coagulans</i>			
35	<i>Staphylococcus sciuri</i>	ATCC 29060		
	subsp. <i>sciuri</i>			
	<i>Staphylococcus simulans</i>	ATCC 27848		
	<i>Staphylococcus warneri</i>	ATCC 27836		
	<i>Staphylococcus xylosus</i>	ATCC 29971		
40	Gram-negative bacteria (n=33)			
	<i>Acinetobacter baumannii</i>	ATCC 19606	<i>Morganella morganii</i>	ATCC 25830
	<i>Bacteroides distasonis</i>	ATCC 8503	<i>Neisseria gonorrhoeae</i>	ATCC 35201
	<i>Bacteroides fragilis</i>	ATCC 25285	<i>Neisseria meningitidis</i>	ATCC 13077
	<i>Bulkholderia cepacia</i>	ATCC 25416	<i>Proteus mirabilis</i>	ATCC 25933
45	<i>Bordetella pertussis</i>	ATCC 9797	<i>Proteus vulgaris</i>	ATCC 13315
	<i>Citrobacter freundii</i>	ATCC 8090	<i>Providencia rettgeri</i>	ATCC 9250
	<i>Enterobacter aerogenes</i>	ATCC 13048	<i>Providencia stuartii</i>	ATCC 29914
	<i>Enterobacter cloacae</i>	ATCC 13047	<i>Pseudomonas aeruginosa</i>	ATCC 27853
	<i>Escherichia coli</i>	ATCC 25922	<i>Pseudomonas fluorescens</i>	ATCC 13525
50	<i>Haemophilus influenzae</i>	ATCC 8907	<i>Salmonella choleraesuis</i>	ATCC 7001
	<i>Haemophilus parahaemolyticus</i>	ATCC 10014	<i>Salmonella typhimurium</i>	ATCC 14028
	<i>Haemophilus parainfluenzae</i>	ATCC 7901	<i>Serratia marcescens</i>	ATCC 8100
	<i>Hafnia alvei</i>	ATCC 13337	<i>Shigella flexneri</i>	ATCC 12022
	<i>Kingella indologenes</i>	ATCC 25869	<i>Shigella sonnei</i>	ATCC 29930
55	<i>Klebsiella oxytoca</i>	ATCC 13182	<i>Stenotrophomonas maltophilia</i>	ATCC 13843
	<i>Klebsiella pneumoniae</i>	ATCC 13883	<i>Yersinia enterocolitica</i>	ATCC 9610
	<i>Moraxella catarrhalis</i>	ATCC 25240		

Table 13. Bacterial species used to test the specificity of the penicillin-resistant *Streptococcus pneumoniae* assay.

	Strain	Reference number	Strain	Reference number
5	Gram-positive species (n=67)			
	<i>Abiotrophia adiacens</i>	ATCC 49175	<i>Staphylococcus hominis</i>	ATCC 27844
	<i>Abiotrophia defectiva</i>	ATCC 49176	<i>Staphylococcus lugdunensis</i>	ATCC 43809
	<i>Actinomyces pyogenes</i>	ATCC 19411	<i>Staphylococcus saprophyticus</i>	ATCC 15305
10	<i>Bacillus anthracis</i>	ATCC 4229	<i>Staphylococcus simulans</i>	ATCC 27848
	<i>Bacillus cereus</i>	ATCC 14579	<i>Staphylococcus. warneri</i>	ATCC 27836
	<i>Bifidobacterium breve</i>	ATCC 15700	<i>Streptococcus acidominimus</i>	ATCC 51726
	<i>Clostridium difficile</i>	ATCC 9689	<i>Streptococcus agalactiae</i>	ATCC 12403
	<i>Enterococcus avium</i>	ATCC 14025	<i>Streptococcus anginosus</i>	ATCC 33397
15	<i>Enterococcus casseliflavus</i>	ATCC 25788	<i>Streptococcus bovis</i>	ATCC 33317
	<i>Enterococcus dispar</i>	ATCC 51266	<i>Streptococcus constellatus</i>	ATCC 27823
	<i>Enterococcus durans</i>	ATCC 19432	<i>Streptococcus cricetus</i>	ATCC 19624
	<i>Enterococcus faecalis</i>	ATCC 29212	<i>Streptococcus cristatus</i>	ATCC 51100
	<i>Enterococcus faecium</i>	ATCC 19434	<i>Streptococcus downei</i>	ATCC 33748
20	<i>Enterococcus flavescens</i>	ATCC 49996	<i>Streptococcus dysgalactiae</i>	ATCC 43078
	<i>Enterococcus gallinarum</i>	ATCC 49573	<i>Streptococcus equi</i>	ATCC 9528
	<i>Enterococcus hirae</i>	ATCC 8043	<i>Streptococcus ferus</i>	ATCC 33477
	<i>Enterococcus mundtii</i>	ATCC 43186	<i>Streptococcus gordonii</i>	ATCC 10558
	<i>Enterococcus raffinosus</i>	ATCC 49427	<i>Streptococcus intermedius</i>	ATCC 27335
25	<i>Lactobacillus lactis</i>	ATCC 19435	<i>Streptococcus mitis</i>	ATCC 903
	<i>Lactobacillus monocytogenes</i>	ATCC 15313	<i>Streptococcus mitis</i>	LSPQ 2583
	<i>Mobiluncus curtisii</i>	ATCC 35242	<i>Streptococcus mitis</i>	ATCC 49456
	<i>Peptococcus niger</i>	ATCC 27731	<i>Streptococcus mutans</i>	ATCC 27175
	<i>Peptostreptococcus acones</i>	ATCC 6919	<i>Streptococcus oralis</i>	ATCC 10557
30	<i>Peptostreptococcus anaerobius</i>	ATCC 27337	<i>Streptococcus oralis</i>	ATCC 9811
	<i>Peptostreptococcus</i>	ATCC 2639	<i>Streptococcus oralis</i>	ATCC 35037
	<i>asaccharolyticus</i>		<i>Streptococcus parasanguinis</i>	ATCC 15912
	<i>Peptostreptococcus lactolyticus</i>	ATCC 51172	<i>Streptococcus parauberis</i>	ATCC 6631
	<i>Peptostreptococcus magnus</i>	ATCC 15794	<i>Streptococcus rattus</i>	ATCC 15912
35	<i>Peptostreptococcus prevotii</i>	ATCC 9321	<i>Streptococcus salivarius</i>	ATCC 7073
	<i>Peptostreptococcus tetradius</i>	ATCC 35098	<i>Streptococcus sanguinis</i>	ATCC10556
	<i>Staphylococcus aureus</i>	ATCC 25923	<i>Streptococcus suis</i>	ATCC 43765
	<i>Staphylococcus capitis</i>	ATCC 27840	<i>Streptococcus uberis</i>	ATCC 19436
	<i>Staphylococcus epidermidis</i>	ATCC 14990	<i>Streptococcus vestibularis</i>	ATCC 49124
40	<i>Staphylococcus haemolyticus</i>	ATCC 29970		
	Gram-negative species (n=33)			
	<i>Actinetobacter baumannii</i>	ATCC 19606	<i>Moraxella morganii</i>	ATCC 13077
	<i>Bordetella pertussis</i>	ATCC 9797	<i>Neisseria gonorrhoeae</i>	ATCC 35201
	<i>Citrobacter diversus</i>	ATCC 27028	<i>Neisseria meningitidis</i>	ATCC 13077
45	<i>Citrobacter freundii</i>	ATCC 8090	<i>Proteus mirabilis</i>	ATCC 25933
	<i>Enterobacter aerogenes</i>	ATCC 13048	<i>Proteus vulgaris</i>	ATCC 13315
	<i>Enterobacter agglomerans</i>	ATCC 27155	<i>Providencia alcalifaciens</i>	ATCC 9886
	<i>Enterobacter cloacae</i>	ATCC 13047	<i>Providencia rettgeri</i>	ATCC 9250
	<i>Escherichia coli</i>	ATCC 25922	<i>Providencia rustigianii</i>	ATCC 33673
50	<i>Haemophilus ducreyi</i>	ATCC 33940	<i>Providencia stuartii</i>	ATCC 33672
	<i>Haemophilus haemolyticus</i>	ATCC 33390	<i>Pseudomonas aeruginosa</i>	ATCC 35554
	<i>Haemophilus influenzae</i>	ATCC 9007	<i>Pseudomonas fluorescens</i>	ATCC 13525
	<i>Haemophilus parainfluenzae</i>	ATCC 7901	<i>Pseudomonas stutzeri</i>	ATCC 17588
	<i>Hafnia alvei</i>	ATCC 13337	<i>Salmonella typhimurium</i>	ATCC 14028
55	<i>Klebsiella oxytoca</i>	ATCC 13182	<i>Serratia marcescens</i>	ATCC 13880
	<i>Klebsiella pneumoniae</i>	ATCC 13883	<i>Shigella flexneri</i>	ATCC 12022
	<i>Moraxella atlantae</i>	ATCC 29525	<i>Yersinia enterocolitica</i>	ATCC 9610
	<i>Moraxella catarrhalis</i>	ATCC 43628		

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Table 14. Bacterial species (n=104) detected by the platelet contaminants assay. Bold characters indicate the major bacterial contaminants found in platelet concentrates.

5	<i>Abiotrophia adiacens</i>	<i>Klebsiella oxytoca</i>	<i>Staphylococcus simulans</i>
	<i>Abiotrophia defectiva</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus warneri</i>
	<i>Acinetobacter baumannii</i>	<i>Legionella pneumophila</i>	<i>Stenotrophomonas maltophilia</i>
	<i>Acinetobacter lwoffii</i>	<i>Megamonas hypermegale</i>	80 <i>Streptococcus acidominimus</i>
	<i>Aerococcus viridans</i>	45 <i>Moraxella atlantae</i>	<i>Streptococcus agalactiae</i>
10	<i>Bacillus anthracis</i>	<i>Moraxella catarrhalis</i>	<i>Streptococcus anginosus</i>
	<i>Bacillus cereus</i>	<i>Morganella morganii</i>	<i>Streptococcus bovis</i>
	<i>Bacillus subtilis</i>	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus constellatus</i>
	<i>Brucella abortus</i>	<i>Neisseria meningitidis</i>	85 <i>Streptococcus cricetus</i>
	<i>Burkholderia cepacia</i>	50 <i>Pasteurella aerogenes</i>	<i>Streptococcus cristatus</i>
15	<i>Citrobacter diversus</i>	<i>Pasteurella multocida</i>	<i>Streptococcus dysgalactiae</i>
	<i>Citrobacter freundii</i>	<i>Peptostreptococcus magnus</i>	<i>Streptococcus equi</i>
	<i>Enterobacter aerogenes</i>	<i>Proteus mirabilis</i>	<i>Streptococcus ferus</i>
	<i>Enterobacter agglomerans</i>	<i>Providencia alcalifaciens</i>	90 <i>Streptococcus gordonii</i>
	<i>Enterobacter cloacae</i>	55 <i>Providencia rettgeri</i>	<i>Streptococcus intermedius</i>
20	<i>Enterococcus avium</i>	<i>Providencia rustigianii</i>	<i>Streptococcus macacae</i>
	<i>Enterococcus casseliflavus</i>	<i>Providencia stuartii</i>	<i>Streptococcus mitis</i>
	<i>Enterococcus dispar</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus mutans</i>
	<i>Enterococcus durans</i>	<i>Pseudomonas fluorescens</i>	95 <i>Streptococcus oralis</i>
	<i>Enterococcus faecalis</i>	60 <i>Pseudomonas stutzeri</i>	<i>Streptococcus parasanguinis</i>
25	<i>Enterococcus faecium</i>	<i>Salmonella bongori</i>	<i>Streptococcus parauberis</i>
	<i>Enterococcus flavescens</i>	<i>Salmonella choleraesuis</i>	<i>Streptococcus pneumoniae</i>
	<i>Enterococcus gallinarum</i>	<i>Salmonella enteritidis</i>	<i>Streptococcus pyogenes</i>
	<i>Enterococcus mundtii</i>	<i>Salmonella gallinarum</i>	100 <i>Streptococcus rattii</i>
	<i>Enterococcus raffinosus</i>	65 <i>Salmonella typhimurium</i>	<i>Streptococcus salivarius</i>
30	<i>Enterococcus solitarius</i>	<i>Serratia liquefaciens</i>	<i>Streptococcus sanguinis</i>
	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	<i>Streptococcus sobrinus</i>
	<i>Gemella morbillorum</i>	<i>Shigella flexneri</i>	<i>Streptococcus uberis</i>
	<i>Haemophilus ducreyi</i>	<i>Shigella sonnei</i>	105 <i>Streptococcus vestibularis</i>
	<i>Haemophilus haemolyticus</i>	70 <i>Staphylococcus aureus</i>	<i>Vibrio cholerae</i>
35	<i>Haemophilus influenzae</i>	<i>Staphylococcus capitis</i>	<i>Yersinia enterocolitica</i>
	<i>Haemophilus</i>	<i>Staphylococcus epidermidis</i>	<i>Yersinia pestis</i>
	<i>parahaemolyticus</i>	<i>Staphylococcus haemolyticus</i>	<i>Yersinia pseudotuberculosis</i>
	<i>Haemophilus parainfluenzae</i>	<i>Staphylococcus hominis</i>	
	<i>Hafnia alvei</i>	75 <i>Staphylococcus lugdunensis</i>	
40	<i>Kingella kingae</i>	<i>Staphylococcus saprophyticus</i>	

Table 15. Microorganism identified by commercial systems¹.

5	<i>Achromobacter</i> species	75	<i>Alcaligenes xylosoxidans</i> subsp. <i>xylosoxidans</i>	150	<i>Brevibacterium</i> species
	<i>Acidaminococcus fermentans</i>		<i>Alloiococcus otitis</i>		<i>Brevundimonas (Pseudomonas) diminuta</i>
	<i>Acinetobacter alcaligenes</i>	80	<i>Anaerobiospirillum succiniciproducens</i>		<i>Brevundimonas (Pseudomonas) vesicularis</i>
	<i>Acinetobacter anitratus</i>		<i>Anaerovibrio lipolytica</i>	155	<i>Brevundimonas</i> species
	<i>Acinetobacter baumannii</i>		<i>Arcanobacterium (Actinomyces) bernardiae</i>		<i>Brochothrix thermosphacta</i>
10	<i>Acinetobacter calcoaceticus</i>		<i>Arcanobacterium (Actinomyces) pyogenes</i>		<i>Brucella abortus</i>
	<i>Acinetobacter calcoaceticus biovar anitratus</i>	85	<i>Arcanobacterium haemolyticum</i>		<i>Brucella canis</i>
	<i>Acinetobacter calcoaceticus biovar lwoffii</i>		<i>Arcobacter cryaerophilus</i>	160	<i>Brucella melitensis</i>
15	<i>Acinetobacter genomospecies</i>		<i>(Campylobacter cryaerophila)</i>		<i>Brucella ovis</i>
	<i>Acinetobacter haemolyticus</i>		<i>Arthrobacter globiformis</i>		<i>Brucella species</i>
	<i>Acinetobacter johnsonii</i>	90	<i>Arthrobacter species</i>		<i>Brucella suis</i>
	<i>Acinetobacter junii</i>		<i>Arxiozyma telluris (Torulopsis pintolopesii)</i>	165	<i>Budvicia aquatica</i>
	<i>Acinetobacter lwoffii</i>		<i>Atopobium minutum (Lactobacillus minutus)</i>		<i>Burkholderia (Pseudomonas) cepacia</i>
20	<i>Acinetobacter radioresistens</i>		<i>Aureobacterium</i> species		<i>Burkholderia (Pseudomonas) gladioli</i>
	<i>Acinetobacter</i> species	95	<i>Bacillus amyloliquefaciens</i>		<i>Burkholderia (Pseudomonas) mallei</i>
	<i>Actinobacillus actinomycetemcomitans</i>		<i>Bacillus anthracis</i>		<i>Burkholderia (Pseudomonas) pseudomallei</i>
	<i>Actinobacillus capsulatus</i>		<i>Bacillus badius</i>	170	<i>Burkholderia species</i>
	<i>Actinobacillus equuli</i>		<i>Bacillus cereus</i>		<i>Buttiauxella agrestis</i>
25	<i>Actinobacillus hominis</i>		<i>Bacillus circulans</i>		<i>Campylobacter coli</i>
	<i>Actinobacillus lignieresii</i>	100	<i>Bacillus coagulans</i>		<i>Campylobacter concisus</i>
	<i>Actinobacillus pleuropneumoniae</i>		<i>Bacillus firmus</i>		<i>Campylobacter fetus</i>
	<i>Actinobacillus species</i>		<i>Bacillus lentus</i>	175	<i>Campylobacter fetus subsp. fetus</i>
	<i>Actinobacillus suis</i>		<i>Bacillus licheniformis</i>		<i>Campylobacter fetus subsp. jejuni</i>
30	<i>Actinobacillus ureae</i>		<i>Bacillus megaterium</i>		<i>Campylobacter fetus subsp. venerealis</i>
	<i>Actinomyces bovis</i>	105	<i>Bacillus mycoides</i>		<i>Campylobacter hyointestinalis</i>
	<i>Actinomyces israelii</i>		<i>Bacillus pantothenicus</i>		<i>Campylobacter jejuni subsp. doylei</i>
	<i>Actinomyces meyeri</i>		<i>Bacillus pumilus</i>	180	<i>Campylobacter jejuni subsp. jejuni</i>
	<i>Actinomyces naeslundii</i>		<i>Bacillus species</i>		<i>Campylobacter lari</i>
35	<i>Actinomyces neuii</i> subsp. <i>anitratus</i>		<i>Bacillus sphaericus</i>		<i>Campylobacter lari subsp. UPTC</i>
	<i>Actinomyces neuii</i> subsp. <i>neuii</i>	110	<i>Bacillus stearothermophilus</i>		<i>Campylobacter mucosalis</i>
	<i>Actinomyces odontolyticus</i>		<i>Bacillus subtilis</i>	185	<i>Campylobacter species</i>
	<i>Actinomyces pyogenes</i>		<i>Bacillus thuringiensis</i>		<i>Campylobacter sputorum</i>
	<i>Actinomyces radlingae</i>		<i>Bacteroides caccae</i>		<i>Campylobacter sputorum subsp. bubulus</i>
40	<i>Actinomyces species</i>		<i>Bacteroides capillosus</i>		<i>Campylobacter sputorum subsp. fecalis</i>
	<i>Actinomyces turicensis</i>	115	<i>Bacteroides distasonis</i>		<i>Campylobacter sputorum subsp. sputorum</i>
	<i>Actinomyces viscosus</i>		<i>Bacteroides eggerthii</i>	190	<i>Campylobacter upsaliensis</i>
	<i>Aerococcus species</i>		<i>Bacteroides fragilis</i>		<i>Candida (Clavispora) lusitanae</i>
	<i>Aerococcus viridans</i>		<i>Bacteroides merdae</i>		<i>Candida (Pichia) guilliermondii</i>
45	<i>Aeromonas caviae</i>		<i>Bacteroides ovatus</i>		<i>Candida (Torulopsis) glabrata</i>
	<i>Aeromonas hydrophila</i>	120	<i>Bacteroides species</i>		<i>Candida albicans</i>
	<i>Aeromonas hydrophila</i> group		<i>Bacteroides splanchnicus</i>	195	<i>Candida boidinii</i>
	<i>Aeromonas jandaei</i>		<i>Bacteroides stercoris</i>		<i>Candida catenulata</i>
	<i>Aeromonas salmonicida</i>		<i>Bacteroides thetaiotaomicron</i>		<i>Candida ciferrii</i>
50	<i>Aeromonas salmonicida</i> subsp. <i>achromogenes</i>		<i>Bacteroides uniformis</i>		<i>Candida colliculosa</i>
	<i>Aeromonas salmonicida</i> subsp. <i>masoucida</i>	125	<i>Bacteroides ureolyticus (B. corrodens)</i>		<i>Candida conglobata</i>
	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i>		<i>Bacteroides vulgatus</i>	200	<i>Candida curvata (Cryptococcus curvatus)</i>
55	<i>Aeromonas schubertii</i>		<i>Bergeyella (Weeksella) zoohelcum</i>		<i>Candida dattila</i>
	<i>Aeromonas sobria</i>	130	<i>Bifidobacterium adolescentis</i>		<i>Candida dubliniensis</i>
	<i>Aeromonas species</i>		<i>Bifidobacterium bifidum</i>		<i>Candida famata</i>
	<i>Aeromonas trota</i>		<i>Bifidobacterium breve</i>	205	<i>Candida globosa</i>
60	<i>Aeromonas veronii</i>		<i>Bifidobacterium dentium</i>		<i>Candida hellenica</i>
	<i>Aeromonas veronii</i> biovar <i>sobria</i>		<i>Bifidobacterium infantis</i>		<i>Candida holmii</i>
	<i>Aeromonas veronii</i> biovar <i>veronii</i>		<i>Bifidobacterium species</i>		<i>Candida humicola</i>
	<i>Agrobacterium radiobacter</i>		<i>Blastoschizomyces (Dipodascus) capitatus</i>		<i>Candida inconspicua</i>
65	<i>Agrobacterium species</i>	135	<i>Bordetella avium</i>	210	<i>Candida intermedia</i>
	<i>Agrobacterium tumefaciens</i>		<i>Bordetella bronchiseptica</i>		<i>Candida kefyr</i>
	<i>Alcaligenes denitrificans</i>		<i>Bordetella parapertussis</i>		<i>Candida krusei</i>
	<i>Alcaligenes faecalis</i>	140	<i>Bordetella pertussis</i>		<i>Candida lambica</i>
	<i>Alcaligenes odorans</i>		<i>Bordetella species</i>		<i>Candida magnoliae</i>
	<i>Alcaligenes odorans (Alcaligenes faecalis)</i>		<i>Borrelia species</i>	215	<i>Candida maris</i>
70	<i>Alcaligenes species</i>		<i>Branhamella (Moraxella) catarrhalis</i>		<i>Candida melibiosica</i>
	<i>Alcaligenes xylosoxidans</i>		<i>Branhamella species</i>		<i>Candida membranaefaciens</i>
	<i>Alcaligenes xylosoxidans</i> subsp. <i>denitrificans</i>	145	<i>Brevibacillus brevis</i>		<i>Candida norvegensis</i>
			<i>Brevibacterium casei</i>	220	<i>Candida norvegica</i>
			<i>Brevibacterium epidermidis</i>		<i>Candida parapsilosis</i>
			<i>Brevibacterium linens</i>		<i>Candida paratropicalis</i>
					<i>Candida pelliculosa</i>

Table 15. Microorganism identified by commercial systems (continued)

<i>Candida pseudotropicalis</i>		<i>Clostridium hastiforme</i>	<i>Corynebacterium urealyticum</i> (group D2)
<i>Candida pulcherrima</i>	80	<i>Clostridium histolyticum</i>	<i>Corynebacterium xerosis</i>
<i>Candida ravautii</i>		<i>Clostridium innocuum</i>	<i>Cryptococcus albidus</i>
<i>Candida rugosa</i>		<i>Clostridium limosum</i>	<i>Cryptococcus ater</i>
5 <i>Candida sake</i>		<i>Clostridium novyi</i>	<i>Cryptococcus cereanus</i>
<i>Candida silvicola</i>		<i>Clostridium novyi A</i>	<i>Cryptococcus gastricus</i>
<i>Candida species</i>	85	<i>Clostridium paraputrificum</i>	<i>Cryptococcus humicolus</i>
<i>Candida sphaerica</i>		<i>Clostridium perfringens</i>	<i>Cryptococcus lactivorius</i>
<i>Candida stellatoidea</i>		<i>Clostridium putrificum</i>	<i>Cryptococcus laurentii</i>
10 <i>Candida tenuis</i>		<i>Clostridium ramosum</i>	<i>Cryptococcus luteolus</i>
<i>Candida tropicalis</i>		<i>Clostridium septicum</i>	<i>Cryptococcus melibiosum</i>
<i>Candida utilis</i>	90	<i>Clostridium sordellii</i>	<i>Cryptococcus neoformans</i>
<i>Candida valida</i>		<i>Clostridium species</i>	<i>Cryptococcus species</i>
<i>Candida vini</i>		<i>Clostridium sphenoides</i>	170 <i>Cryptococcus terreus</i>
15 <i>Candida viswanathii</i>		<i>Clostridium sporogenes</i>	<i>Cryptococcus uniguttulatus</i>
<i>Candida zeylanoides</i>		<i>Clostridium subterminale</i>	<i>Debaryomyces hansenii</i>
<i>Capnocytophaga gingivalis</i>	95	<i>Clostridium tertium</i>	<i>Debaryomyces marama</i>
<i>Capnocytophaga ochracea</i>		<i>Clostridium tetani</i>	<i>Debaryomyces polymorphus</i>
<i>Capnocytophaga species</i>		<i>Clostridium tyrobutyricum</i>	175 <i>Debaryomyces species</i>
20 <i>Capnocytophaga sputigena</i>		<i>Comamonas (Pseudomonas) acidovorans</i>	<i>Dermabacter hominis</i>
<i>Cardiobacterium hominis</i>		100 <i>Comamonas (Pseudomonas) testosteroni</i>	<i>Dermacoccus (Micrococcus) nishinomiyaensis</i>
<i>Camobacterium divergens</i>		<i>Comamonas species</i>	180 <i>Dietzia species</i>
<i>Camobacterium piscicola</i>		<i>Corynebacterium accolens</i>	<i>Edwardsiella hoshinae</i>
CDC group ED-2		<i>Corynebacterium atermantans</i>	<i>Edwardsiella ictaluri</i>
25 CDC group EF4 (<i>Pasteurella</i> sp.)		<i>Corynebacterium amycolatum</i>	<i>Edwardsiella species</i>
CDC group EF-4A		<i>Corynebacterium aquaticum</i>	<i>Edwardsiella tarda</i>
CDC group EF-4B	105	<i>Corynebacterium argenteorotense</i>	185 <i>Eikenella corrodens</i>
CDC group EQ-Z		<i>Corynebacterium auris</i>	<i>Empedobacter brevis</i> (<i>Flavobacterium breve</i>)
CDC group HB-5		<i>Corynebacterium bovis</i>	<i>Enterobacter aerogenes</i>
30 CDC group II K-2		110 <i>Corynebacterium coyleae</i>	<i>Enterobacter agglomerans</i>
CDC group IV C-2 (<i>Bordetella</i> -like)		<i>Corynebacterium cystitidis</i>	190 <i>Enterobacter amnigenus</i>
CDC group M5		<i>Corynebacterium diphtheriae</i>	<i>Enterobacter amnigenus asburiae</i>
CDC group M6		<i>Corynebacterium diphtheriae</i> biotype <i>belfanti</i>	(CDC enteric group 17)
35 <i>Cedecea davisae</i>		115 <i>Corynebacterium diphtheriae</i> biotype <i>gravis</i>	<i>Enterobacter amnigenus biogroup 1</i>
<i>Cedecea lapagei</i>		<i>Corynebacterium diphtheriae</i> biotype <i>intermedius</i>	<i>Enterobacter amnigenus biogroup 2</i>
<i>Cedecea neteri</i>		120 <i>Corynebacterium diphtheriae</i> biotype <i>mitis</i>	195 <i>Enterobacter asburiae</i>
<i>Cedecea species</i>		<i>Corynebacterium diphtheriae</i> biotype <i>mitis</i>	<i>Enterobacter cancerogenus</i>
<i>Cellulomonas (Oerskovia) turbata</i>		<i>Corynebacterium flavescens</i>	<i>Enterobacter cloacae</i>
<i>Cellulomonas species</i>		<i>Corynebacterium glucuronolyticum</i>	<i>Enterobacter gergoviae</i>
40 <i>Chlamydia species</i>		<i>Corynebacterium glucuronolyticum-seminale</i>	<i>Enterobacter hormaechei</i>
<i>Chromobacterium violaceum</i>		125 <i>Corynebacterium group A</i>	200 <i>Enterobacter intermedius</i>
<i>Chryseobacterium (Flavobacterium) indologenes</i>		<i>Corynebacterium group A-4</i>	<i>Enterobacter sakazakii</i>
<i>Chryseobacterium (Flavobacterium) meningosepticum</i>		<i>Corynebacterium group A-5</i>	<i>Enterobacter species</i>
<i>Chryseobacterium gleum</i>		<i>Corynebacterium group ANF</i>	<i>Enterobacter taylorae</i>
<i>Chryseobacterium species</i>		<i>Corynebacterium group B</i>	<i>Enterobacter taylorae</i> (CDC enteric group 19)
<i>Chryseomonas indologenes</i>		130 <i>Corynebacterium group B-3</i>	<i>Enterococcus (Streptococcus) cecorum</i>
50 <i>Citeromyces matritensis</i>		<i>Corynebacterium group F</i>	<i>Enterococcus (Streptococcus) faecalis</i> (Group D)
<i>Citrobacter amalonaticus</i>		<i>Corynebacterium group F-1</i>	<i>Enterococcus (Streptococcus) faecium</i> (Group D)
<i>Citrobacter braakii</i>		<i>Corynebacterium group F-2</i>	<i>Enterococcus (Streptococcus) saccharolyticus</i>
<i>Citrobacter diversus</i>		<i>Corynebacterium group G</i>	<i>Enterococcus avium</i> (Group D)
<i>Citrobacter farmeri</i>		135 <i>Corynebacterium group G-1</i>	215 <i>Enterococcus casseliflavus</i> (<i>Streptococcus faecium</i> subsp. <i>casseliflavus</i>)
<i>Citrobacter freundii</i>		<i>Corynebacterium group G-2</i>	<i>Enterococcus durans</i> (<i>Streptococcus faecium</i> subsp. <i>durans</i>) (Group D)
55 <i>Citrobacter freundii</i> complex		<i>Corynebacterium group I</i>	<i>Enterococcus gallinarum</i>
<i>Citrobacter koseri</i>		140 <i>Corynebacterium jeikeium</i> (group JK)	<i>Enterococcus hirae</i>
<i>Citrobacter sedlakii</i>		<i>Corynebacterium kutscheri</i> (<i>C. murium</i>)	<i>Enterococcus malodoratus</i>
<i>Citrobacter species</i>		<i>Corynebacterium macginleyi</i>	<i>Enterococcus mundtii</i>
<i>Citrobacter werkmanii</i>		<i>Corynebacterium minutissimum</i>	<i>Enterococcus raffinosus</i>
60 <i>Citrobacter youngae</i>		<i>Corynebacterium pilosum</i>	<i>Enterococcus species</i>
<i>Clostridium acetobutylicum</i>		145 <i>Corynebacterium propinquum</i>	<i>Erwinia amylovora</i>
<i>Clostridium barati</i>		<i>Corynebacterium pseudodiphtheriticum</i>	<i>Erwinia carotovora</i>
<i>Clostridium beijerinckii</i>		<i>Corynebacterium pseudotuberculosis</i>	<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>
<i>Clostridium bifementans</i>		150 <i>Corynebacterium pyogenes</i>	<i>Erwinia carotovora</i> subsp. <i>betavascularum</i>
65 <i>Clostridium botulinum</i>		<i>Corynebacterium renale</i>	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>
<i>Clostridium botulinum</i> (NP) B&F		<i>Corynebacterium renale</i> group	<i>Erwinia chrysanthemi</i>
<i>Clostridium botulinum</i> (NP) E		<i>Corynebacterium seminale</i>	<i>Erwinia cypripedii</i>
<i>Clostridium botulinum</i> (P) A&H		<i>Corynebacterium species</i>	<i>Erwinia mallotivora</i>
<i>Clostridium botulinum</i> (P) F		155 <i>Corynebacterium striatum</i> (<i>C. flavidum</i>)	
70 <i>Clostridium botulinum</i> G1		<i>Corynebacterium ulcerans</i>	
<i>Clostridium botulinum</i> G2			
<i>Clostridium butyricum</i>			
<i>Clostridium cadaveris</i>			
<i>Clostridium chauvoei</i>			
75 <i>Clostridium clostridioforme</i>			
<i>Clostridium difficile</i>			
<i>Clostridium fallax</i>			
<i>Clostridium glycolicum</i>			

Table 15. Microorganism identified by commercial systems (continued)

<i>Erwinia nigrifluens</i>	VII	<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i>
<i>Erwinia quercina</i>	80 <i>Haemophilus parainfluenzae</i> biotype	<i>Lactobacillus pentosus</i>
<i>Erwinia rhapontici</i>	VIII	<i>Lactobacillus plantarum</i>
<i>Erwinia rubrifaciens</i>	<i>Haemophilus paraphrohaemolyticus</i>	<i>Lactobacillus salivarius</i>
5 <i>Erwinia salicis</i>	<i>Haemophilus paraphrophilus</i>	<i>Lactobacillus salivarius</i> var. <i>salicinius</i>
<i>Erwinia species</i>	<i>Haemophilus segnis</i>	<i>Lactobacillus species</i>
<i>Erysipelothrix rhusiopathiae</i>	85 <i>Haemophilus somnus</i>	<i>Lactococcus diacetylactis</i>
<i>Erysipelothrix species</i>	<i>Haemophilus species</i>	<i>Lactococcus garvieae</i>
<i>Escherichia blattae</i>	<i>Hafnia alvei</i>	165 <i>Lactococcus lactis</i> subsp. <i>cremoris</i>
10 <i>Escherichia coli</i>	<i>Hanseniaspora guilliermondii</i>	<i>Lactococcus lactis</i> subsp. <i>diacetylactis</i>
<i>Escherichia coli</i> A-D	<i>Hanseniaspora uvarum</i>	<i>Lactococcus lactis</i> subsp. <i>hordniae</i>
<i>Escherichia coli</i> O157:H7	90 <i>Hanseniaspora valbyensis</i>	<i>Lactococcus lactis</i> subsp. <i>lactis</i>
<i>Escherichia fergusonii</i>	<i>Hansenula anomala</i>	<i>Lactococcus plantarum</i>
<i>Escherichia hermannii</i>	<i>Hansenula holstii</i>	170 <i>Lactococcus raffinolactis</i>
15 <i>Escherichia species</i>	<i>Hansenula polymorpha</i>	<i>Leclercia adecarboxylata</i>
<i>Escherichia vulneris</i>	<i>Helicobacter (Campylobacter) cinaedi</i>	<i>Legionella species</i>
<i>Eubacterium aerofaciens</i>	95 <i>Helicobacter (Campylobacter)</i>	<i>Leminorella species</i>
<i>Eubacterium alactolyticum</i>	<i>fennelliae</i>	<i>Leptospira species</i>
<i>Eubacterium lentum</i>	<i>Helicobacter (Campylobacter) pylori</i>	175 <i>Leptotrichia buccalis</i>
20 <i>Eubacterium limosum</i>	<i>Issatchenkia orientalis</i>	<i>Leuconostoc (Weissella)</i>
<i>Eubacterium species</i>	<i>Kingella denitrificans</i>	<i>paramesenteroides</i>
<i>Ewingella americana</i>	100 <i>Kingella indologenes</i>	<i>Leuconostoc carnosum</i>
<i>Filobasidiella neoformans</i>	<i>Kingella kingae</i>	180 <i>Leuconostoc citreum</i>
<i>Filobasidium floriforme</i>	<i>Kingella species</i>	<i>Leuconostoc gelidum</i>
25 <i>Filobasidium uniguttulatum</i>	<i>Klebsiella ornithinolytica</i>	<i>Leuconostoc lactis</i>
<i>Flavimonas oryzihabitans</i>	<i>Klebsiella oxytoca</i>	<i>Leuconostoc mesenteroides</i>
<i>Flavobacterium gleum</i>	105 <i>Klebsiella planticola</i>	<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>
<i>Flavobacterium indologenes</i>	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	185 <i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>
<i>Flavobacterium odoratum</i>	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>
30 <i>Flavobacterium species</i>	110 <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>	190 <i>Leuconostoc species</i>
<i>Francisella novicida</i>	<i>Klebsiella species</i>	<i>Listeria grayi</i>
<i>Francisella philomiragia</i>	<i>Klebsiella terrigena</i>	<i>Listeria innocua</i>
<i>Francisella species</i>	<i>Kloëckera apiculata</i>	<i>Listeria ivanovii</i>
<i>Francisella tularensis</i>	115 <i>Kloëckera apis</i>	<i>Listeria monocytogenes</i>
35 <i>Fusobacterium mortiferum</i>	<i>Kloëckera japonica</i>	195 <i>Listeria murrayi</i>
<i>Fusobacterium necrogenes</i>	<i>Kloëckera species</i>	<i>Listeria seeligeri</i>
<i>Fusobacterium necrophorum</i>	<i>Kluyvera ascorbata</i>	<i>Listeria species</i>
<i>Fusobacterium nucleatum</i>	<i>Kluyvera cryocrescens</i>	<i>Listeria welshimeri</i>
<i>Fusobacterium species</i>	120 <i>Kluyvera species</i>	200 <i>Megasphaera elsdenii</i>
40 <i>Fusobacterium varium</i>	<i>Kluyveromyces lactis</i>	<i>Methylobacterium mesophilicum</i>
<i>Gaffkya species</i>	<i>Kluyveromyces marxianus</i>	<i>Metschnikowia pulcherrima</i>
<i>Gardnerella vaginalis</i>	<i>Kluyveromyces thermotolerans</i>	<i>Microbacterium species</i>
<i>Gemella haemolysans</i>	<i>Kocuria (Micrococcus) kristinae</i>	<i>Micrococcus luteus</i>
45 <i>Gemella morbillorum</i>	125 <i>Kocuria (Micrococcus) rosea</i>	<i>Micrococcus lylae</i>
<i>Geotrichum candidum</i>	<i>Kocuria (Micrococcus) varians</i>	205 <i>Micrococcus species</i>
<i>Geotrichum fermentans</i>	<i>Koserella trabusli</i>	<i>Mobiluncus curtisii</i>
<i>Geotrichum penicillarum</i>	<i>Kytococcus (Micrococcus) sedentarius</i>	<i>Mobiluncus mulleris</i>
<i>Geotrichum penicillatum</i>	<i>Lactobacillus (Weissella) viridescens</i>	<i>Mobiluncus species</i>
50 <i>Geotrichum species</i>	130 <i>Lactobacillus A</i>	<i>Moellerella wisconsensis</i>
<i>Gordona species</i>	<i>Lactobacillus acidophilus</i>	210 <i>Moraxella (Branhamella) catarrhalis</i>
<i>Haemophilus aegyptius</i>	<i>Lactobacillus B</i>	<i>Moraxella atlantae</i>
<i>Haemophilus aphrophilus</i>	<i>Lactobacillus brevis</i>	<i>Moraxella bovis</i>
<i>Haemophilus ducreyi</i>	<i>Lactobacillus buchneri</i>	<i>Moraxella lacunata</i>
55 <i>Haemophilus haemoglobinophilus</i>	135 <i>Lactobacillus casei</i>	<i>Moraxella nonliquefaciens</i>
<i>Haemophilus haemolyticus</i>	<i>Lactobacillus casei</i> subsp. <i>casei</i>	215 <i>Moraxella osloensis</i>
<i>Haemophilus influenzae</i>	<i>Lactobacillus casei</i> subsp. <i>lactosus</i>	<i>Moraxella phenylpyruvica</i>
<i>Haemophilus influenzae</i> biotype I	<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>	<i>Moraxella species</i>
<i>Haemophilus influenzae</i> biotype II	<i>Lactobacillus cateniformis</i>	<i>Morganella morganii</i>
60 <i>Haemophilus influenzae</i> biotype III	140 <i>Lactobacillus cellobiosus</i>	<i>Morganella morganii</i> subsp. <i>morganii</i>
<i>Haemophilus influenzae</i> biotype IV	<i>Lactobacillus collinoides</i>	220 <i>Morganella morganii</i> subsp. <i>sibonii</i>
<i>Haemophilus influenzae</i> biotype V	<i>Lactobacillus coprophilus</i>	<i>Mycobacterium africanum</i>
<i>Haemophilus influenzae</i> biotype VI	<i>Lactobacillus crispatus</i>	<i>Mycobacterium asiaticum</i>
65 <i>Haemophilus influenzae</i> biotype VII	<i>Lactobacillus curvatus</i>	<i>Mycobacterium avium</i>
<i>Haemophilus influenzae</i> biotype VIII	145 <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	<i>Mycobacterium bovis</i>
<i>Haemophilus paragaillinarum</i>	<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i>	225 <i>Mycobacterium chelonae</i>
<i>Haemophilus parahaemolyticus</i>	<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	<i>Mycobacterium fortuitum</i>
<i>Haemophilus parainfluenzae</i>	150 <i>Lactobacillus fermentum</i>	<i>Mycobacterium gordonae</i>
<i>Haemophilus parainfluenzae</i> biotype I	<i>Lactobacillus fructivorans</i>	<i>Mycobacterium kansasii</i>
<i>Haemophilus parainfluenzae</i> biotype II	<i>Lactobacillus helveticus</i>	<i>Mycobacterium malmoeense</i>
<i>Haemophilus parainfluenzae</i> biotype III	<i>Lactobacillus helveticus</i> subsp. <i>jugurti</i>	230 <i>Mycobacterium marinum</i>
<i>Haemophilus parainfluenzae</i> biotype IV	155 <i>Lactobacillus jensenii</i>	<i>Mycobacterium phlei</i>
75 <i>Haemophilus parainfluenzae</i> biotype V	<i>Lactobacillus lindneri</i>	<i>Mycobacterium scrofulaceum</i>
<i>Haemophilus parainfluenzae</i> biotype VI	<i>Lactobacillus minutus</i>	<i>Mycobacterium smegmatis</i>
<i>Haemophilus parainfluenzae</i> biotype		<i>Mycobacterium species</i>

Table 15. Microorganisms identified by commercial systems (continued)¹.

		60	<i>Streptococcus</i> Gamma (non)-hemolytic	120	<i>Tetragenococcus</i> (<i>Pediococcus</i>) <i>halophilus</i>
	<i>Staphylococcus hyicus</i>		<i>Streptococcus gordonii</i>		<i>Torulaspora delbrueckii</i>
	<i>Staphylococcus intermedius</i>		<i>Streptococcus</i> Group B		(<i>Saccharomyces rosei</i>)
	<i>Staphylococcus kloosii</i>		<i>Streptococcus</i> Group C		<i>Torulopsis candida</i>
5	<i>Staphylococcus lentus</i>		<i>Streptococcus</i> Group D		<i>Torulopsis haemulonii</i>
	<i>Staphylococcus lugdunensis</i>	65	<i>Streptococcus</i> Group E	125	<i>Torulopsis inconspicua</i>
	<i>Staphylococcus saprophyticus</i>		<i>Streptococcus</i> Group F		<i>Treponema</i> species
	<i>Staphylococcus schleiferi</i>		<i>Streptococcus</i> Group G		<i>Trichosporon asahii</i>
	<i>Staphylococcus sciuri</i>		<i>Streptococcus</i> Group L		<i>Trichosporon asteroides</i>
10	<i>Staphylococcus simulans</i>		<i>Streptococcus</i> Group P		<i>Trichosporon beigelii</i>
	<i>Staphylococcus species</i>	70	<i>Streptococcus</i> Group U	130	<i>Trichosporon cutaneum</i>
	<i>Staphylococcus warneri</i>		<i>Streptococcus intermedius</i>		<i>Trichosporon inkin</i>
	<i>Staphylococcus xylosum</i>		<i>Streptococcus intermedius</i> (<i>Streptococcus milleri</i> II)		<i>Trichosporon mucoides</i>
	<i>Stenotrophomonas</i> (<i>Xanthomonas</i>) <i>maltophilis</i>		75		<i>Trichosporon ovoides</i>
15	<i>Stephanoascus ciferrii</i>		<i>Streptococcus intermedius</i> (<i>Streptococcus</i>)	135	<i>Trichosporon pullulans</i>
	<i>Stomatococcus mucilaginosus</i>		<i>Streptococcus milleri</i> group		<i>Trichosporon species</i>
	<i>Streptococcus acidominimus</i>		<i>Streptococcus mitis</i>		<i>Turicella otitidis</i>
	<i>Streptococcus agalactiae</i>		<i>Streptococcus mitis</i> (<i>Streptococcus</i>)		<i>Ureaplasma</i> species
20	<i>Streptococcus agalactiae</i> (Group B)	80	<i>Streptococcus mutans</i>	140	<i>Ureaplasma urealyticum</i>
	<i>Streptococcus agalactiae</i> hemolytic		<i>Streptococcus mutans</i> (<i>Streptococcus</i>)		<i>Veillonella parvula</i> (<i>V. alcalescens</i>)
	<i>Streptococcus agalactiae</i> non-hemolytic		<i>Streptococcus mutans</i> (<i>Streptococcus</i>)		<i>Veillonella</i> species
	<i>Streptococcus alactolyticus</i>		<i>Streptococcus oralis</i>		<i>Vibrio alginolyticus</i>
25	<i>Streptococcus anginosus</i>	85	<i>Streptococcus parasanguis</i>	145	<i>Vibrio cholerae</i>
	<i>Streptococcus anginosus</i> (Group D, nonenterococci)		<i>Streptococcus pneumoniae</i>		<i>Vibrio damsela</i>
	<i>Streptococcus beta-hemolytic</i> group A		<i>Streptococcus porcinus</i>		<i>Vibrio fluvialis</i>
	<i>Streptococcus beta-hemolytic</i> non-group A or B		<i>Streptococcus pyogenes</i>		<i>Vibrio furnissii</i>
30	<i>Streptococcus beta-hemolytic</i> non-group A	90	<i>Streptococcus pyogenes</i> (Group A)	150	<i>Vibrio harveyi</i>
	<i>Streptococcus beta-hemolytic</i>		<i>Streptococcus salivarius</i>		<i>Vibrio hollisae</i>
	<i>Streptococcus bovis</i> (Group D, nonenterococci)		<i>Streptococcus salivarius</i> (<i>Streptococcus</i>)		<i>Vibrio metschnikovii</i>
35	<i>Streptococcus bovis</i> I	95	<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>		<i>Vibrio mimicus</i>
	<i>Streptococcus bovis</i> II		<i>Streptococcus sanguis</i>		<i>Vibrio parahaemolyticus</i>
	<i>Streptococcus canis</i>		<i>Streptococcus sanguis</i> I (<i>Streptococcus</i>)		<i>Vibrio species</i>
	<i>Streptococcus constellatus</i>		<i>Streptococcus sanguis</i> II (<i>Streptococcus</i>)		<i>Vibrio species</i> SF
40	<i>Streptococcus constellatus</i> (<i>Streptococcus milleri</i> I)	100	<i>Streptococcus sanguis</i> II (<i>Streptococcus</i>)	160	<i>Vibrio vulnificus</i>
	<i>Streptococcus constellatus</i> (<i>Streptococcus</i>)		<i>Streptococcus sobrinus</i>		<i>Weeksella</i> (<i>Bergeyella</i>) <i>virosa</i>
	<i>Streptococcus downei</i>		<i>Streptococcus species</i>	155	<i>Weeksella</i> species
45	<i>Streptococcus dysgalactiae</i> subsp. <i>dysgalactiae</i>	105	<i>Streptococcus suis</i> I		<i>Weeksella virosa</i>
	<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>		<i>Streptococcus suis</i> II		<i>Williopsis</i> (<i>Hansenula</i>) <i>satumus</i>
	<i>Streptococcus equi</i> (Group C/Group G)		<i>Streptococcus uberis</i>		<i>Xanthomonas campestris</i>
50	<i>Streptococcus</i>		<i>Streptococcus uberis</i> (<i>Streptococcus</i>)	165	<i>Xanthomonas</i> species
	<i>Streptococcus equi</i> subsp. <i>equi</i>	110	<i>Streptococcus vestibularis</i>		<i>Yarrowia</i> (<i>Candida</i>) <i>lipolytica</i>
	<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>		<i>Streptococcus zooepidemicus</i>		<i>Yersinia aldovae</i>
	<i>Streptococcus equinus</i>		<i>Streptococcus zooepidemicus</i> (Group C)		<i>Yersinia enterocolitica</i>
55	<i>Streptococcus equinus</i> (Group D, nonenterococci)	115	<i>Streptomyces somaliensis</i>	170	<i>Yersinia enterocolitica</i> group
	<i>Streptococcus equisimilis</i>		<i>Streptomyces species</i>		<i>Yersinia frederiksenii</i>
	<i>Streptococcus equisimilis</i> (Group C/Group G <i>Streptococcus</i>)		<i>Suttonella</i> (<i>Kingella</i>) <i>indologenes</i>	175	<i>Yersinia intermedia</i>
			<i>Tatumella ptyseos</i>		<i>Yersinia intermedius</i>
					<i>Yersinia kristensenii</i>
					<i>Yersinia pestis</i>
					<i>Yersinia pseudotuberculosis</i>
					<i>Yersinia pseudotuberculosis</i> SF
					<i>Yersinia ruckeri</i>
					<i>Yersinia species</i>
					<i>Yokenella regensburgei</i>
					<i>Yokenella regensburgei</i> (<i>Koserella trabulsi</i>)
					<i>Zygoascus hellenicus</i>
					<i>Zygosaccharomyces</i> species

¹ The list includes microorganisms that may be identified by API identification test systems and VITEK[®] automated identification system from bioMérieux Inc., or by the MicroScan[®] - WalkAway[®] automated systems from Dade Behring. Identification relies on classical identification methods using batteries of biochemical and other phenotypic tests.

Table 16. *tuf* gene sequences obtained in our laboratory (Example 42).

Species	Strain no.	Gene	GenBank Accession no.*
<i>Abiotrophia adiacens</i>	ATCC49175	<i>tuf</i>	AF124224
<i>Enterococcus avium</i>	ATCC14025	<i>tufA</i>	AF124220
		<i>tufB</i>	AF274715
<i>Enterococcus casseliflavus</i>	ATCC25788	<i>tufA</i>	AF274716
		<i>tufB</i>	AF274717
<i>Enterococcus cecorum</i>	ATCC43198	<i>tuf</i>	AF274718
<i>Enterococcus columbae</i>	ATCC51263	<i>tuf</i>	AF274719
<i>Enterococcus dispar</i>	ATCC51266	<i>tufA</i>	AF274720
		<i>tufB</i>	AF274721
<i>Enterococcus durans</i>	ATCC19432	<i>tufA</i>	AF274722
		<i>tufB</i>	AF274723
<i>Enterococcus faecalis</i>	ATCC29212	<i>tuf</i>	AF124221
<i>Enterococcus faecium</i>	ATCC 19434	<i>tufA</i>	AF124222
		<i>tufB</i>	AF274724
<i>Enterococcus gallinarum</i>	ATCC49573	<i>tufA</i>	AF124223
		<i>tufB</i>	AF274725
<i>Enterococcus hirae</i>	ATCC8043	<i>tufA</i>	AF274726
		<i>tufB</i>	AF274727
<i>Enterococcus malodoratus</i>	ATCC43197	<i>tufA</i>	AF274728
		<i>tufB</i>	AF274729
<i>Enterococcus mundtii</i>	ATCC43186	<i>tufA</i>	AF274730
		<i>tufB</i>	AF274731
<i>Enterococcus pseudoavium</i>	ATCC49372	<i>tufA</i>	AF274732
		<i>tufB</i>	AF274733
<i>Enterococcus raffinosus</i>	ATCC49427	<i>tufA</i>	AF274734
		<i>tufB</i>	AF274735
<i>Enterococcus saccharolyticus</i>	ATCC43076	<i>tuf</i>	AF274736
<i>Enterococcus solitarius</i>	ATCC49428	<i>tuf</i>	AF274737
<i>Enterococcus sulfureus</i>	ATCC49903	<i>tuf</i>	AF274738
<i>Lactococcus lactis</i>	ATCC11154	<i>tuf</i>	AF274745
<i>Listeria monocytogenes</i>	ATCC15313	<i>tuf</i>	AF274746
<i>Listeria seeligeri</i>	ATCC35967	<i>tuf</i>	AF274747
<i>Staphylococcus aureus</i>	ATCC25923	<i>tuf</i>	AF274739
<i>Staphylococcus epidermidis</i>	ATCC14990	<i>tuf</i>	AF274740
<i>Streptococcus mutans</i>	ATCC25175	<i>tuf</i>	AF274741
<i>Streptococcus pneumoniae</i>	ATCC6303	<i>tuf</i>	AF274742
<i>Streptococcus pyogenes</i>	ATCC19615	<i>tuf</i>	AF274743
<i>Streptococcus suis</i>	ATCC43765	<i>tuf</i>	AF274744

*Corresponding sequence ID NO. for the above ATCC strains are given in table 7.

Table 17. tuf gene sequences selected from databases for Example 42.

Species	Gene	Accession no.*
<i>Agrobacterium tumefaciens</i>	tufA	X99673
	tufB	X99674
<i>Anacystis nidulans</i>	tuf	X17442
<i>Aquifex aeolicus</i>	tufA	AE000657
	tufB	AE000657
<i>Bacillus stearothermophilus</i>	tuf	AJ000260
<i>Bacillus subtilis</i>	tuf	AL009126
<i>Bacteroides fragilis</i>	tuf	P33165
<i>Borrelia burgdorferi</i>	tuf	AE000783
<i>Brevibacterium linens</i>	tuf	X76863
<i>Bulkholderia cepacia</i>	tuf	P33167
<i>Campylobacter jejuni</i>	tufB	Y17167
<i>Chlamydia pneumoniae</i>	tuf	AE001363
<i>Chlamydia trachomatis</i>	tuf	M74221
<i>Corynebacterium glutamicum</i>	tuf	X77034
<i>Cytophaga lytica</i>	tuf	X77035
<i>Deinococcus radiodurans</i>	tuf	AE000513
<i>Escherichia coli</i>	tufA	J01690
	tufB	J01717
<i>Fervidobacterium islandicum</i>	tuf	Y15788
<i>Haemophilus influenzae</i>	tufA	L42023
	tufB	L42023
<i>Helicobacter pylori</i>	tuf	AE000511
<i>Homo sapiens</i> (Human)	EF-1 α	X03558
<i>Methanococcus jannaschii</i>	EF-1 α	U67486
<i>Mycobacterium leprae</i>	tuf	D13869
<i>Mycobacterium tuberculosis</i>	tuf	X63539
<i>Mycoplasma genitalium</i>	tuf	L43967
<i>Mycoplasma pneumoniae</i>	tuf	U00089
<i>Neisseria gonorrhoeae</i>	tufA	L36380
<i>Nicotiana tabacum</i> (Tobacco)	EF-1 α	U04632
<i>Peptococcus niger</i>	tuf	X76869
<i>Planobispora rosea</i>	tuf1	U67308
<i>Saccharomyces cerevisiae</i> (Yeast)	EF-1 α	X00779
<i>Salmonella typhimurium</i>	tufA	X55116
	tufB	X55117
<i>Shewanella putrefaciens</i>	tuf	P33169
<i>Spirochaeta aurantia</i>	tuf	X76874
<i>Spirulina platensis</i>	tufA	X15646
<i>Streptomyces aureofaciens</i>	tuf1	AF007125
<i>Streptomyces cinnamomeus</i>	tuf1	X98831
<i>Streptomyces coelicolor</i>	tuf1	X77039
	tuf3	X77040
<i>Streptomyces collinus</i>	tuf1	S79408
<i>Streptomyces ramocissimus</i>	tuf1	X67057
	tuf2	X67058
	tuf3	X67059
<i>Synechocystis</i> sp.	tuf	AB001339
<i>Taxobacter ocellatus</i>	tuf	X77036
<i>Thermotoga maritima</i>	tuf	AE000512
<i>Thermus aquaticus</i>	tuf	X66322
<i>Thermus thermophilus</i>	tuf	X06657
<i>Thiobacillus cuprinus</i>	tuf	U78300
<i>Treponema pallidum</i>	tuf	AE000520
<i>Wolinella succinogenes</i>	tuf	X76872

* Sequence data were obtained from GenBank, EMBL, and SWISSPROT databases. Genes were designated as appeared in the references.

Table 18. Nucleotide and amino acid sequence identities of EF-Tu between different enterococci and other low G+C gram-positive bacteria.

The upper right triangle represents the deduced amino acid sequence identities of gram-positive bacterial EF-Tu, while the lower left triangle represents the DNA sequence identities of the corresponding *tuf* genes. The sequence identities between different enterococcal *tufA* genes are boxed while those between enterococcal *tufB* genes are shaded.

Bacterial <i>tuf</i> gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
1. <i>E. avium</i> <i>tufA</i>		96	98	96	96	96	96	97	95	98	99	95	95	96	94	96	93	86	87	85	86	86	86	86	85	86	87	86	92	91	90	90	90	92	84	85	84	82	83	
2. <i>E. casseliflavus</i> <i>tufA</i>	90		97	96	96	99	96	95	96	96	96	95	95	96	96	94	93	87	88	88	87	87	86	87	87	87	88	88	84	81	90	91	91	92	86	87	85	85	85	
3. <i>E. dispar</i> <i>tufA</i>	93	90		95	95	96	95	96	95	97	97	91	90	95	95	93	86	87	85	87	87	86	87	86	87	86	87	87	89	80	89	90	90	92	85	86	84	85	84	
4. <i>E. durans</i> <i>tufA</i>	88	90	89		98	96	99	93	99	95	96	90	91	94	95	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	90	90	90	91	85	86	84	84	84	
5. <i>E. faecium</i> <i>tufA</i>	88	90	89	96		96	98	93	98	95	96	89	91	88	94	93	92	87	88	86	86	87	87	86	87	87	88	87	94	92	91	91	91	91	83	85	86	84	84	
6. <i>E. gallinarum</i> <i>tufA</i>	90	97	89	89	89		96	93	95	96	96	88	89	89	96	93	92	87	87	86	87	87	87	87	86	87	87	88	87	93	92	90	90	90	93	85	86	84	83	84
7. <i>E. hirae</i> <i>tufA</i>	90	90	89	89	86	89		93	99	95	96	91	91	89	95	96	92	86	87	88	88	86	85	86	86	87	87	87	94	90	90	90	90	90	91	85	86	84	84	84
8. <i>E. malodoratus</i> <i>tufA</i>	96	91	94	90	89	90	89		92	97	97	89	89	90	93	96	92	86	85	82	85	85	85	85	85	85	86	86	92	90	88	88	89	91	83	84	83	83	82	
9. <i>E. mundtii</i> <i>tufA</i>	89	89	88	96	93	89	96	88		94	95	88	90	88	94	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	89	90	90	91	85	86	84	84	84	
10. <i>E. pseudoavium</i> <i>tufA</i>	97	92	93	90	89	91	89	97	89		98	90	90	90	91	95	96	94	87	87	86	87	87	86	87	87	88	87	93	91	89	90	90	91	85	86	84	85	84	
11. <i>E. raffinosus</i> <i>tufA</i>	97	91	93	90	89	89	89	97	88	97		91	90	90	94	96	93	88	87	85	86	86	85	86	85	87	87	93	89	89	90	89	91	84	85	84	84	83		
12. <i>E. oecorum</i> <i>tufA</i>	90	90	95	96	96	95	96	92	95	95	95		96	95	93	93	93	88	88	87	87	86	86	86	89	87	89	89	93	90	90	91	91	93	86	86	84	85	84	
13. <i>E. columbae</i> <i>tufA</i>	90	90	95	96	97	96	96	93	95	95	95	97		95	94	92	92	89	88	86	87	88	88	87	87	87	89	89	94	92	91	91	92	83	86	86	85	86	85	
14. <i>E. laecocis</i> <i>tufA</i>	91	91	90	89	96	97	94	94	94	95	96	90	89		94	94	93	87	88	87	87	86	86	87	87	87	88	87	93	91	89	90	91	93	86	86	85	85	85	
15. <i>E. saccharolyticus</i> <i>tufA</i>	91	91	91	80	87	90	89	91	89	92	91	89	89	82		94	92	86	87	85	87	86	84	86	85	87	87	92	90	89	89	88	90	84	85	84	84	84	84	
16. <i>E. sulfuricus</i> <i>tufA</i>	91	89	90	91	88	88	90	91	89	92	91	89	89	81	94		91	85	84	81	84	85	84	84	84	81	84	85	85	91	90	87	88	89	91	82	83	82	82	
17. <i>E. solentis</i> <i>tuf</i>	83	84	83	83	84	83	82	84	83	84	84	84	83	84	83	83		88	87	86	87	86	87	86	87	88	88	88	89	92	91	89	90	90	91	86	85	85	85	84
18. <i>E. avium</i> <i>tufB</i>	77	77	78	78	76	77	78	78	77	78	77	78	78	78	77	76	77		93	93	94	94	94	92	98	93	99	97	87	86	87	86	85	86	89	88	87	85	86	
19. <i>E. casseliflavus</i> <i>tufB</i>	71	72	72	72	70	72	72	70	71	72	72	72	70	72	72	68	72	79		93	95	95	96	95	93	85	84	94	87	86	88	88	84	85	90	90	89	88	88	
20. <i>E. dispar</i> <i>tufB</i>	76	78	77	77	77	77	77	76	77	77	77	77	77	77	78	75	78	82	79		91	91	92	91	94	92	93	93	86	83	85	85	82	84	89	89	87	87	86	
21. <i>E. durans</i> <i>tufB</i>	77	78	78	78	78	77	78	77	78	77	78	77	77	77	78	75	75	83	80	82		98	95	97	94	97	95	94	87	86	88	88	84	85	90	91	89	88	89	
22. <i>E. faecium</i> <i>tufB</i>	76	75	76	76	75	77	76	76	76	75	76	77	77	77	76	74	74	80	78	79	86		96	97	95	95	94	87	87	88	88	84	86	90	90	89	87	87		
23. <i>E. gallinarum</i> <i>tufB</i>	72	73	72	73	72	74	72	71	72	72	72	72	72	73	73	72	72	78	81	77	81	82		94	94	95	95	94	85	87	89	89	84	86	90	90	89	87	88	
24. <i>E. hirae</i> <i>tufB</i>	75	74	75	75	75	75	75	76	75	75	74	74	74	74	75	72	74	80	79	79	84	83	79		93	87	93	94	87	85	86	88	83	85	89	90	88	87	87	
25. <i>E. malodoratus</i> <i>tufB</i>	76	76	76	77	77	77	77	74	77	76	76	77	75	77	77	73	78	90	79	83	81	80	77	79		93	98	97	87	86	87	87	85	86	88	89	87	85	86	
26. <i>E. mundtii</i> <i>tufB</i>	74	74	74	75	73	74	74	74	74	74	74	74	74	75	74	71	73	80	80	78	85	85	80	84	80		94	87	86	88	88	84	86	90	90	89	88	89		
27. <i>E. pseudoavium</i> <i>tufB</i>	77	77	78	77	76	78	77	77	76	78	77	77	78	78	77	78	91	80	85	84	81	79	80	91	80		96	87	88	87	85	87	88	84	86	90	89	88	87	
28. <i>E. raffinosus</i> <i>tufB</i>	78	79	79	78	77	77	78	78	77	79	79	78	78	78	79	77	90	79	84	84	81	77	80	90	81	92		87	85	87	88	84	86	90	89	88	88	87		
29. <i>E. adhaerens</i> <i>tuf</i>	88	87	87	86	86	86	86	88	88	88	87	88	88	88	89	82	77	70	76	77	76	71	73	77	73	78	78		90	88	89	90	91	85	86	84	85	83		
30. <i>B. subtilis</i> <i>tuf</i>	81	80	79	79	80	80	79	79	79	80	81	80	81	81	81	80	78	78	73	69	73	73	71	70	71	72	71	74	74	78		91	92	90	90	82	82	83	82	84
31. <i>L. monocytogenes</i> <i>tuf</i>	82	81	82	82	82	82	81	81	81	82	81	81	81	81	81	79	79	76	71	75	75	73	74	75	73	78	76	79	82		99	88	90	84	84	84	84	84	84	
32. <i>L. seeligen</i> <i>tuf</i>	84	81	82	82	82	81	82	81	82	81	82	81	82	80	80	81	79	75	69	75	75	73	69	72	74	72	74	74	83	79	81		96	81	82	82	80	82		
33. <i>S. aureus</i> <i>tuf</i>	84	84	83	83	83	84	84	82	84	83	84	86	86	84	82	81	79	75	69	75	75	73	69	72	74	72	74	75	81	79	82	81	94		83	83	83	83	83	
34. <i>S. epidermidis</i> <i>tuf</i>	83	85	83	84	83	84	84	82	84	83	83	86	87	85	83	82	79	75	69	75	75	73	68	72	74	72	74	74	83	79	81		96	81	82	82	80	82		
35. <i>S. mutans</i> <i>tuf</i>	77	77	76	77	77	77	76	75	76	76	76	77	76	76	74	78	79	72	77	78	77	74	75	78	75	78	81	77	75	76	77	76	74	74		96	96	99		
36. <i>S. pneumoniae</i> <i>tuf</i>	78	77	76	77	77	77	75	78	76	76	77	76	77	76	75	74	75	76	72	76	78	76	73	74	77	75	75	78	75	76	77	76	76	73	72	87		94	89	
37. <i>S. pyogenes</i> <i>tuf</i>	76	77	76	77	76	75	77	74	77	78	75	76	75	75	75	73	75	74	71	75	78	75	73	74	75	75	75	77	76	77	76	76	73	72	87		93	94	89	
38. <i>S. suis</i> <i>tuf</i>	74	78	76	74	75	76	74	78	76	77	77	75	78	76	73	75	74	71	75	78	74	70	74	75	73	73	77	77	77	77	77	77	72	73	88	93		88		
39. <i>L. lactis</i> <i>tuf</i>	75	76	75	76	75	75	76	75	76	76	76	77	75	78	75	72	74	75	72	75	77	76	71	75	74	75	75	75	75	75	75	77	76	74	74	80	83	82	81	

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Table 19. Strains analyzed in Example 43.

Taxon	Strain*	Strain†	16S rDNA sequence accession number
<i>Cedecea</i>	ATCC 33431 ^T		
<i>Cedecea lapagei</i>	ATCC 33432 ^T		
<i>Cedecea neteri</i>	ATCC 33855 ^T		
<i>Citrobacter amalonaticus</i>	ATCC 25405 ^T	CDC 9020-77 ^T	AF025370
<i>Citrobacter braakii</i>	ATCC 43162		
		CDC 080-58 ^T	AF025368
<i>Citrobacter farmeri</i>	ATCC 51112 ^T	CDC 2991-81 ^T	AF025371
<i>Citrobacter freundii</i>	ATCC 8090 ^T	DSM 30039 ^T	AJ233408
<i>Citrobacter koseri</i>	ATCC 27156 ^T		
<i>Citrobacter sedlakii</i>	ATCC 51115 ^T	CDC 4696-86 ^T	AF025364
<i>Citrobacter werkmanii</i>	ATCC 51114 ^T	CDC 0876-58 ^T	AF025373
<i>Citrobacter youngae</i>	ATCC 29935 ^T		
<i>Edwardsiella hoshinae</i>	ATCC 33379 ^T		
<i>Edwardsiella tarda</i>	ATCC 15947 ^T		
		CDC 4411-68	AF015259
<i>Enterobacter aerogenes</i>	ATCC 13048 ^T	JCM 1235 ^T	AB004750
<i>Enterobacter agglomerans</i>	ATCC 27989		
<i>Enterobacter amnigenus</i>	ATCC 33072 ^T	JCM 1237 ^T	AB004749
<i>Enterobacter asburiae</i>	ATCC 35953 ^T	JCM 6051 ^T	AB004744
<i>Enterobacter cancerogenus</i>	ATCC 35317 ^T		
<i>Enterobacter cloacae</i>	ATCC 13047 ^T		
<i>Enterobacter gergoviae</i>	ATCC 33028 ^T	JCM 1234 ^T	AB004748
<i>Enterobacter hormaechei</i>	ATCC 49162 ^T		
<i>Enterobacter sakazakii</i>	ATCC 29544 ^T	JCM 1233 ^T	AB004746
<i>Escherichia coli</i>	ATCC 11775 ^T	ATCC 11775 ^T	X80725
<i>Escherichia coli</i>	ATCC 25922	ATCC 25922	X80724
<i>Escherichia coli</i> (ETEC)	ATCC 35401		
<i>Escherichia coli</i> (O157:H7)	ATCC 43895	ATCC 43895	Z83205
<i>Escherichia fergusonii</i>	ATCC 35469 ^T		
<i>Escherichia hermanii</i>	ATCC 33650 ^T		
<i>Escherichia vulneris</i>	ATCC 33821 ^T	ATCC 33821 ^T	X80734
<i>Ewingella americana</i>	ATCC 33852 ^T		
		NCPPB 3905	X88848
<i>Hafnia alvei</i>	ATCC 13337 ^T	ATCC 13337 ^T	M59155
<i>Klebsiella omithinolytica</i>	ATCC 31898		
		CIP 103.364	U78182
<i>Klebsiella oxytoca</i>	ATCC 33496		
		ATCC 13182 ^T	U78183
<i>Klebsiella planticola</i>	ATCC 33531 ^T	JCM 7251 ^T	AB004755
<i>Klebsiella pneumoniae</i>			
subsp. <i>pneumoniae</i>	ATCC 13883 ^T	DSM 30104 ^T	AJ233420
subsp. <i>ozaenae</i>	ATCC 11296 ^T	ATCC 11296 ^T	Y17654
subsp. <i>rhinoscleromatis</i>	ATCC 13884 ^T		

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Table 19. Strains analyzed in Example 43 (continued).

Taxon	Strain*	Strain†	16S rDNA sequence accession number
<i>Kluyvera ascorbata</i>	ATCC 33433 ^T	ATCC 14236	Y07650
<i>Kluyvera cryocrescens</i>	ATCC 33435 ^T		
<i>Kluyvera georgiana</i>	ATCC 51603 ^T		
<i>Leclercia adecarboxylata</i>	ATCC 23216 ^T		
<i>Leminorella grimontii</i>	ATCC 33999 ^T	DSM 5078 ^T	AJ233421
<i>Moellerella wisconsensis</i>	ATCC 35017 ^T		
<i>Morganella morganii</i>	ATCC 25830 ^T		
<i>Pantoea agglomerans</i>	ATCC 27155 ^T	DSM 3493 ^T	AJ233423
<i>Pantoea dispersa</i>	ATCC 14589 ^T		
<i>Plesiomonas shigelloides</i>	ATCC 14029 ^T		
<i>Pragia fontium</i>	ATCC 49100 ^T	DSM 5563 ^T	AJ233424
<i>Proteus mirabilis</i>	ATCC 25933		
<i>Proteus penneri</i>	ATCC 33519 ^T		
<i>Proteus vulgaris</i>	ATCC 13315 ^T	DSM 30118 ^T	AJ233425
<i>Providencia alcalifaciens</i>	ATCC 9886 ^T		
<i>Providencia rettgeri</i>	ATCC 9250		
<i>Providencia rustigianii</i>	ATCC 33673 ^T		
<i>Providencia stuartii</i>	ATCC 33672		
<i>Rahnella aquatilis</i>	ATCC 33071 ^T	DSM 4594 ^T	AJ233426
<i>Salmonella choleraesuis</i>			
subsp. <i>arizonae</i>	ATCC 13314 ^T		
subsp. <i>choleraesuis</i>			
serotype Choleraesuis	ATCC 7001		
serotype Enteritidis‡	ATCC 13076 ^T		
		SE22	SE22
serotype Gallinarum	ATCC 9184		
serotype Heidelberg	ATCC 8326		
serotype Paratyphi A	ATCC 9150		
serotype Paratyphi B	ATCC 8759		
serotype Typhi‡	ATCC 10749		
		St111	U88545
serotype Typhimurium‡	ATCC 14028		
serotype Virchow	ATCC 51955		
subsp. <i>dianzonae</i>	ATCC 43973 ^T		
subsp. <i>houtenae</i>	ATCC 43974 ^T		
subsp. <i>indica</i>	ATCC 43976 ^T		
subsp. <i>salamae</i>	ATCC 43972 ^T		
<i>Serratia fonticola</i>	DSM 4576 ^T	DSM 4576 ^T	AJ233429
<i>Serratia grimesii</i>	ATCC 14460 ^T	DSM 30063 ^T	AJ233430
<i>Serratia liquefaciens</i>	ATCC 27592 ^T		
<i>Serratia marcescens</i>	ATCC 13880 ^T	DSM 30121 ^T	AJ233431
<i>Serratia odorifera</i>	ATCC 33077 ^T	DSM 4582 ^T	AJ233432
<i>Serratia plymuthica</i>	DSM 4540 ^T	DSM 4540 ^T	AJ233433
<i>Serratia rubidaea</i>	DSM 4480 ^T	DSM 4480 ^T	AJ233436
<i>Shigella boydii</i>	ATCC 9207	ATCC 9207	X96965
<i>Shigella dysenteriae</i>	ATCC 11835		
		ATCC 13313 ^T	X96966
		ATCC 25931	X96964

Table 19. Strains analyzed in Example 43 (continued).

Taxon	Strain*	Strain†	16S rDNA sequence accession number
<i>Shigella flexneri</i>	ATCC 12022	ATCC 12022	X96963
<i>Shigella sonnei</i>	ATCC 29930 ^T		
<i>Tatumella pyseos</i>	ATCC 33301 ^T	DSM 5000 ^T	AJ233437
<i>Trabulsiella guamensis</i>	ATCC 49490 ^T		
<i>Yersinia enterocolitica</i>	ATCC 9610 ^T	ATCC 9610 ^T	M59292
<i>Yersinia frederiksenii</i>	ATCC 33641 ^T		
<i>Yersinia intermedia</i>	ATCC 29909 ^T		
<i>Yersinia pestis</i>	RRB KIMD27		
		ATCC 19428 ^T	X75274
<i>Yersinia pseudotuberculosis</i>	ATCC 29833 ^T		
<i>Yersinia rohdei</i>	ATCC 43380 ^T	ER-2935 ^T	X75276
<i>Shewanella putrefaciens</i>	ATCC 8071 ^T		
<i>Vibrio cholerae</i>	ATCC 25870		
		ATCC 14035 ^T	X74695

T Type strain

5 *Strains used in this study for sequencing of partial *tuf* and *atpD* genes. SEQ ID NOs. for *tuf* and *atpD* sequences corresponding to the above reference strains are given in table 7.

†Strains used in other studies for sequencing of 16S rDNA gene. When both strain numbers are on the same row, both strains are considered to be the same although strain numbers may be different.

‡Phylogenetic serotypes considered species by the Bacteriological Code (1990 Revision).

Table 20. PCR primer pairs used in this study

Primer SEQ ID NO.	Sequence	Nucleotide positions*	Amplicon length (bp)
<i>tuf</i>			
664	5'-AAYATGATIACIGGIGCIGCICARATGGA- 3'	271-299	884
697	5'-CCIACIGTICKICCRCCYTCRCG-3'	1132-1156	
<i>atpD</i>			
568	5'-RTIATIGGIGCIGTIRTIGAYGT-3'	25-47	884
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	
700	5'-TIRTIGAYGTCGARTTCCCTCARG-3'	38-61	871
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	

- 5 *The nucleotide positions given are for *E. coli tuf* and *atpD* sequences (GenBank accession no. AE000410 and V00267, respectively). Numbering starts from the first base of the initiation codon.

Table 21. Selection of *M. catarrhalis*-specific primer pairs from SEQ ID NO: 29¹ (466 pb DNA fragment) other than those previously tested².

Primer	Sequence	Amplicon size (bp)	<i>Moraxella catarrhalis</i> ATCC 43628	<i>Moraxella catarrhalis</i> ATCC 53879	<i>Moraxella nonliquefaciens</i>	<i>Moraxella lacunata</i>	<i>Moraxella osloensis</i>	<i>Moraxella atlantae</i>	<i>Moraxella phenylpyruvica</i>	<i>Kingella indologenes</i>	<i>Kingella kingae</i>	<i>Neisseria meningitidis</i>	<i>Neisseria gonorrhoeae</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
SEQ ID NO:118	CGCTGACGGCTTGTGTACCA	118	+	+	-	-	-	-	-	-	-	-	-	-	-
SEQ ID NO:119	TGTTTGTAGCTTTTATTTTGA														
VBmcat1	TGCTTAAGATTTCACCTCTGCCATTTT	93	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat2	TAAGTCGCTGACGGCTTGT														
VBmcat3	CCTGCACCAACAGTCATCAT	140	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat4	AATTCACCAACAATGTCAAAGC														
VBmcat5	AATGATAACCAAGTCAAGCAAGC	219	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat6	GGTGCATGGTGATTTGTAAAA														
VBmcat7	GTGTGCGTTTCACTTTTACAAAT	160	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat8	GGTGTAAAGCTGATGATGAGAG														
VBmcat9	TGACCATGCACACCCCTTATT	167	+	+	-	-	-	-	-	-	-	-	-	-	-
VBmcat10	TCATTGGGATGAAAGTATCGTT														

¹ SEQ ID NO. from US patent 6,001,564.² All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55°C and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.³ All positive results showed a strong amplification signal with genomic DNA from the target species *M. catarrhalis*.

Table 22. Selection of *S. epidermidis*-specific primer pairs from SEQ ID NO: 36¹ (705 pb DNA fragment) other than those previously tested.

Primer	Sequence (all 25 nucleotides)	Amplicon size (bp)	<i>Staphylococcus epidermidis</i> ATCC 14990	<i>Staphylococcus epidermidis</i> ATCC 12228	<i>Staphylococcus capitis</i>	<i>Staphylococcus cohnii</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus auricularis</i>	<i>Staphylococcus</i>	<i>Staphylococcus hominis</i>	<i>Staphylococcus</i>	<i>Staphylococcus simulans</i>	<i>Staphylococcus warneri</i>	<i>Bacillus subtilis</i>	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>	<i>Enterococcus gallinarum</i>	<i>Listeria monocytogenes</i>	<i>Streptococcus agalactiae</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pyogenes</i>	Annealing temperature ² (°C)
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	125	3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep3	CATAGTCTGATTGCTCAAAAGTCTTG				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep4	GCGAATAGTGAACATACATCTGTTG	208	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
VBsep5	CACGCTCTTTTGCAATTTCCATTGA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep6	GAAGCAAATATCAAAATGCACCAG				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65
VBsep7	AAAGTCTTTTGTCTTCTCAGATTCA	177	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep8	GTGTTACACAGGATGATGCTCTTA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
VBsep9	GAGCATCCATACCTGTGAACACAGA				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65
VBsep10	TTTTCCTCAATTACAAAGAGACATCAGT	153	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55
VBsep11	TTTGAATTCGCATGTACTTTGTTTG				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
VBsep12	CCCCGGTTCGAAATCGATAAAAAG				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65
		135	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55

¹ SEQ ID NO. from US patent 6,001,564.² All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55 to 65°C and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.³ All positive results showed a strong amplification signal with genomic DNA from the target species *S. epidermidis*. The intensity of the positive amplification signal with species other than *S. epidermidis* was variable.

NT = not tested.

Table 23. Influence of nucleotide variation(s) on the efficiency of the PCR amplification: Example with SEQ ID NO: 146 from *S. epidermidis*.

Primer ¹	Sequence (all 25 nucleotides)	Number of mutation	<i>Staphylococcus epidermidis</i> ² ATCC 14990					<i>Staphylococcus aureus</i>
			50°C	55°C			50°C	
			1	1	0,1	0,01	1	
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	0						
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA	0	3 ⁴	3+	2+	+		-
VBmut1	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut2	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut3	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut4	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut5	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut6	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut7	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut8	CAAAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+		-
VBmut9	CAAAAGAGCGTGGAGAAAAGTATCA	2	3+	3+	2+	+		-
VBmut10	CAAAAGAGCGTGGAGAAAAGTATCA	2	3+	3+	2+	+		-
VBmut11	CAAAAGAGCGTGGAGAAAAGTATCA	2	3+	3+	2+	+		-
VBmut12	CAAAAGAGCGTGGAGAAAAGTATCA	3	3+	3+	2+	+		-
VBmut13	CAAAAGAGCGTGGAGAAAAGTATCA	4	3+	2+	+	-		-

¹ All PCR tests were performed with SEQ ID NO:145 without modification combined with SEQ ID NO:146 or 13 modified versions of SEQ ID NO:146. Boxed nucleotides indicate changes in SEQ ID NO:146. All SEQ ID NOs. are from US patent 6,001,564.

² The tests with *S. epidermidis* were performed by using an annealing temperature of 55°C with 1, 0,1 and 0,01 ng of purified genomic DNA or at 50°C with 1 ng of purified genomic DNA.

³ The tests with *S. aureus* were performed only at 50°C with 1 ng of genomic DNA.

⁴ The intensity of the positive amplification signal was quantified as follows: 3+ = strong signal, 2+ = intermediate signal and + = weak signal.

Table 24. Effect of the primer length on the efficiency of the PCR amplification¹: Example with the AT-rich SEQ ID NO: 145² and SEQ ID NO: 146² from *S. epidermidis*.

Primer	Sequence	Length (nt)	45°C						55°C						Staphylococcus aureus ⁴		Staphylococcus haemolyticus		Staphylococcus capitis		Staphylococcus warneri	
			1			0,1			0,01			1										
			1	0,1	0,01	1	0,1	0,01	1	0,1	0,01	1	0,1	0,01	1	0,1	0,01	45	55	45	55	45
VBsep301	ATATCATCAAAAAGTTGGCGAACCTTTTCA	30																				
VBsep302	AATTGCAAAAGAGCGTGGAGAAAAGTATCA	30																				
SEQ ID NO:145	ATCAAAAAGTTGGCGAACCTTTTCA	25																				
SEQ ID NO:146	CAAAAGAGCGTGGAGAAAAGTATCA	25																				
VBsep201	AAAGTTGGCGAACCTTTTCA	20																				
VBsep202	GAGCGTGGAGAAAAGTATCA	20																				
VBsep171	GTTGGCGAACCTTTTCA	17																				
VBsep172	CGTGGAGAAAAGTATCA	17																				
VBsep151	TGGCGAACCTTTTCA	15																				
VBsep152	TGGAGAAAAGTATCA	15																				

¹ All PCR tests were performed using an annealing temperature of 45 or 55°C and 30 cycles of amplification.

² All SEQ ID NOs. in this Table are from US patent 6,001,546.

³ The tests with *S. epidermidis* were made with 1, 0,1 and 0,01 ng of purified genomic DNA.

⁴ The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.

⁵ The intensity of the positive amplification signal was quantified as follows: 4+ = very strong signal, 3+ = strong signal, 2+ = intermediate signal and + = weak signal.

NT = not tested.

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Table 25. Effect of the primer length on the efficiency of the PCR amplification¹: Example with the GC-rich SEQ ID NO: 83² and SEQ ID NO: 84² from *P. aeruginosa*.

Primer	Sequence	Length (nt)	1	0,1	0,01	<i>Pseudomonas fluorescens</i> ⁴	<i>Burkholderia cepacia</i>	<i>Shewanella putida</i>	<i>Stenotrophomonas maltophilia</i>	<i>Neisseria meningitidis</i>	<i>Haemophilus parahaemolyticus</i>
SEQ ID NO 83	CGAGCGGTGGTGTTCATC	19	2 ⁵	+	-	.	.	.	.	.	.
SEQ ID NO 84	CAAGTCGTCGTCGAGGGA	19									
Pse554-16a	CGAGCGGTGGTGTTC	16	2+	+	-	.	.	.	.	.	.
Pse674-16a	GTCGTCGTCGAGGGA	16									
Pse554-13b	GCGGGTGGTGTTC	13	2+	+	-	.	.	.	.	.	.
Pse674-13a	GTCGTCGAGGGA	13									

¹ All PCR tests were performed using an annealing temperature of 55°C and 30 cycles of amplification.

² All SEQ ID NOs. in this Table are from US patent 6,001,546.

³ The tests with *P. aeruginosa* were made with 1, 0,1 and 0,01 ng of purified genomic DNA.

⁴ The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.

⁵ The intensity of the positive amplification signal was quantified as follows: 2+ = strong signal and + = moderately strong signal.

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Annex I: Specific and ubiquitous primers for nucleic acid amplification (tuf sequences).

			Originating DNA fragment	
5	SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
10	Bacterial species: <i>Acinetobacter baumannii</i>			
	1692	5'-GGT GAG AAC TGT GGT ATC TTA CTT	1	478-501
	1693 ^a	5'-CAT TTC AAC GCC TTC TTT CAA CTG	1	691-714
15	Bacterial species: <i>Chlamydia pneumoniae</i>			
	630	5'-CGG AGC TAT CCT AGT CGT TTC A	20	2-23
	629 ^a	5'-AAG TTC CAT CTC AAC AAG GTC AAT A	20	146-170
20	2085	5'-CAA ACT AAA GAA CAT ATC TTG CTA	20	45-68
	2086 ^a	5'-ATA TAA TTT GCA TCA CCT TCA AG	20	237-259
	2087	5'-TCA GCT CGT GGG ATT AGG AGA G	20	431-452
	2088 ^a	5'-AGG CTT CAC GCT GTT AGG CTG A	20	584-605
25	Bacterial species: <i>Chlamydia trachomatis</i>			
	554	5'-GTT CCT TAC ATC GTT GTT TTT CTC	22	82-105
	555 ^a	5'-TCT CGA ACT TTC TCT ATG TAT GCA	22	249-272
30	Parasitical species: <i>Cryptosporidium parvum</i>			
	798	5'-TGG TTG TCC CAG CCG ATC GTT T	865	158-179
	804 ^a	5'-CCT GGG ACG GCC TCT GGC AT	865	664-683
35	799	5'-ACC TGT GAA TAC AAG CAA TCT	865	280-300
	805 ^a	5'-CTC TTG TCC ATC TTA GCA GT	865	895-914
	800	5'-GAT GAA ATC TTC AAC GAA GTT GAT	865	307-330
40	806 ^a	5'-AGC ATC ACC AGA CTT GAT AAG	865	946-966
	801	5'-ACA ACA CCG AGA AGA TCC CA	865	353-372
	803 ^a	5'-ACT TCA GTG GTA ACA CCA GC	865	616-635
45	802	5'-TTG CCA TTT CTG GTT TCG TT	865	377-396
	807 ^a	5'-AAA GTG GCT TCA AAG GTT GC	865	981-1000
	Bacterial species: <i>Enterococcus faecium</i>			
50	1696	5'-ATG TTC CTG TAG TTG CTG GA	64	189-208
	1697 ^a	5'-TTT CTT CAG CAA TAC CAA CAA C	64	422-443
	Bacterial species: <i>Klebsiella pneumoniae</i>			
55	1329	5'-TGT AGA GCG CGG TAT CAT CAA AGT A	103	352-377
	1330 ^a	5'-AGA TTC GAA CTT GGT GTG CGG G	103	559-571

^a These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

Claims

1. A method for detecting the presence or absence in a sample of at least one microorganism belonging to the *Streptococcus* genus comprising:

- i) Contacting the sample with at least one oligonucleotide selected to be sufficiently complementary to hybridize to at least one *tuf* target nucleic acid specific to said genus;
- ii) Allowing said oligonucleotide and said *tuf* nucleic acid to hybridize under conditions such that said oligonucleotide hybridizes to said *tuf* nucleic acid, wherein said oligonucleotide hybridizes only to target nucleic acids from microorganisms belonging to said genus and does not detectably hybridize to *tuf* nucleic acids from non-target microorganisms; and
- iii) Testing for hybridization of said oligonucleotide to said *tuf* target nucleic acid,

wherein said at least one oligonucleotide is capable of hybridizing to each of the *tuf* target nucleic acids consisting of SEQ ID NOs: 206-231, 971, 977, 979 and 982-986 or complements thereof; and

wherein said oligonucleotide is capable of:

- a. specifically amplifying its target nucleic acids using PCR conditions comprising for each amplification cycle a denaturation step of 1 s at 95°C and an annealing-extension step of 30 s at 50-65°C; or
- b. specifically hybridizing to its target nucleic acids under the following conditions:
 - hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:
 - aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and
 - bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

2. The method of claim 1, wherein said at least one oligonucleotide comprises an oligonucleotide which specifically hybridizes to a nucleotide sequence defined by SEQ ID NO: 547, 548 or 1202 or a complement thereof.

3. The method of claim 2, wherein said at least one oligonucleotide comprises a first primer consisting of a nucleotide sequence defined by SEQ ID NO: 547 and a second primer consisting of a nucleotide sequence defined by SEQ ID NO: 548.

4. The method of any one of claims 1 to 3, wherein said *Streptococcus* genus consists of *S. agalactiae*, *S. acidominimus*, *S. anginosus*, *S. bovis*, *S. cricetus*, *S. cristatus*, *S. downei*, *S. dysgalactiae*, *S. equi*, *S. ferus*, *S. gordonii*, *S. macacae*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. pneumoniae*, *S. rattii*, *S. sanguinis*, *S. sobrinus*, *S. suis*, *S. uberis*, and *S. vestibularis*.
5. The method of any one of claims 1 to 4, further comprising contacting the sample with:
 - a) at least one species-specific oligonucleotide which hybridizes to *Streptococcus agalactiae tuf* nucleic acid sequences as set forth in SEQ ID NOs: 207 to 210 or complements thereof; and/or
 - b) at least one species-specific oligonucleotide which hybridizes to *Streptococcus agalactiae atpD* nucleic acid sequences as set forth in SEQ ID NOs: 379-383 or complements thereof.
6. The method of any one of claims 1 to 5, wherein step (iii) is performed by using a nucleic acid target amplification method.
7. The method of any one of claims 1 to 5, wherein step (iii) is based on a signal amplification method.
8. The method of claim 6, wherein said amplification method is PCR.
9. The method of any one of claims 1 to 6 and 8, wherein a plurality of primers and/or probes are used in a multiplex PCR assay.
10. The method of any one of claims 1 to 9, wherein said oligonucleotide has at least 12 nucleotides in length.
11. An isolated oligonucleotide primer or probe of at least 12 nucleotides in length which hybridizes specifically to each of the target nucleic acids defined by SEQ ID NOs: 206-231, 982-985, 971, 977, 979 and 986 or complements thereof for detecting the presence or absence in a sample of at least one microorganism belonging to the *Streptococcus* genus, wherein said oligonucleotide is capable of:

- a. specifically amplifying its target nucleic acids using PCR conditions comprising for each amplification cycle a denaturation step of 1 s at 95°C and an annealing-extension step of 30 s at 50-65°C ; or
- b. specifically hybridizing to its target nucleic acids under the following conditions:
 - hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:
 - aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and
 - bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

12. An isolated *tuf* oligonucleotide primer or probe comprising at least 12 consecutive nucleotides of the nucleotide sequence defined in SEQ ID NO: 547 or 548, or a complementary sequence thereof, wherein said *tuf* oligonucleotide primer or probe is less than 100 nucleotides long.

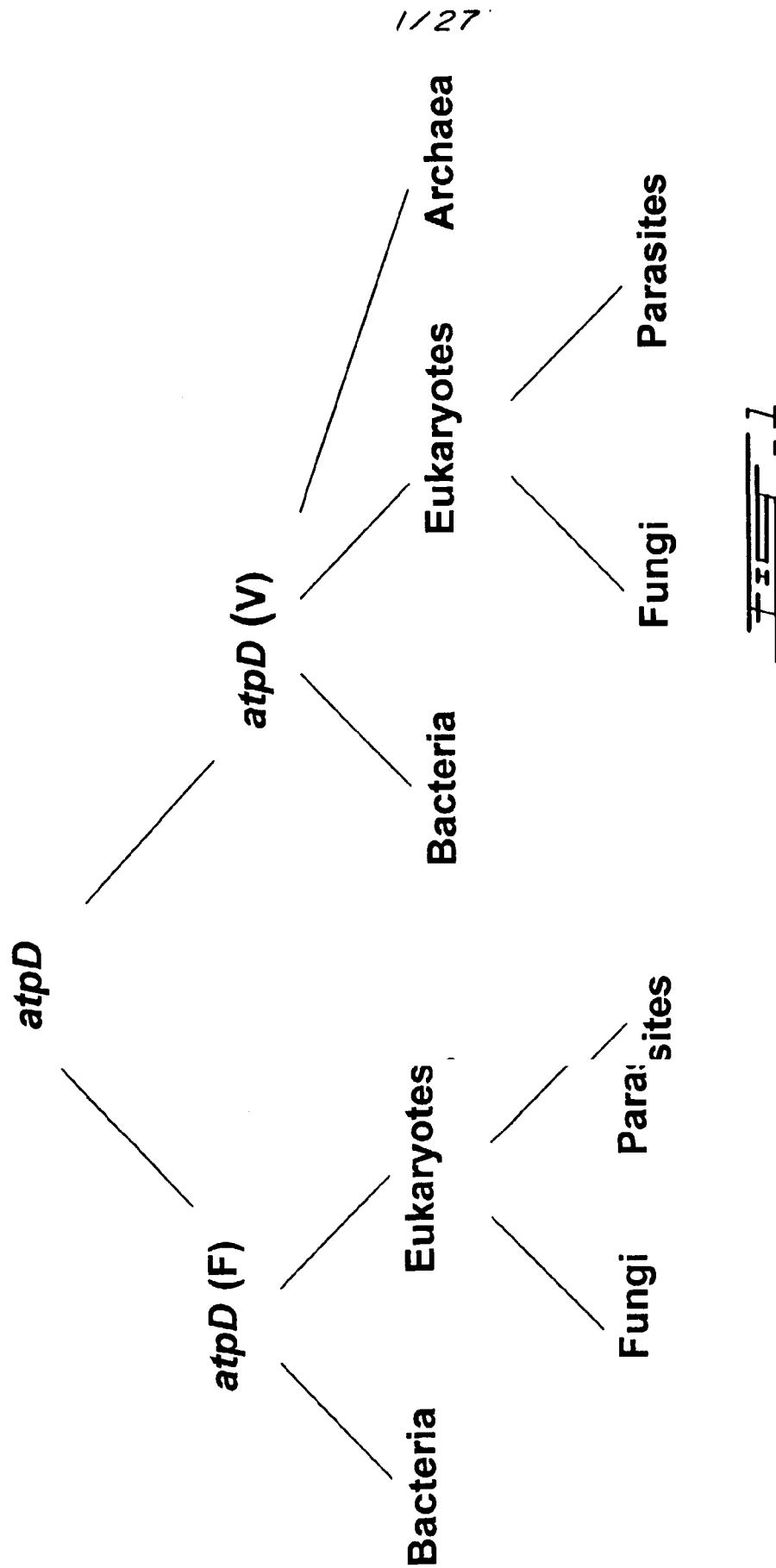
13. The isolated oligonucleotide of claim 11 or 12, wherein said oligonucleotide consists of 12-30 nucleotides in length.

14. A diagnostic kit for detecting the presence or absence in a sample of at least one microorganism belonging to the *Streptococcus* genus, comprising any suitable combination of genus-specific oligonucleotide probes and/or primers of at least 12 nucleotides in length which hybridize specifically to each of the target nucleic acids defined by SEQ ID NOs: 206-231, 982-985, 971, 977, 979 and 986 or complements thereof,

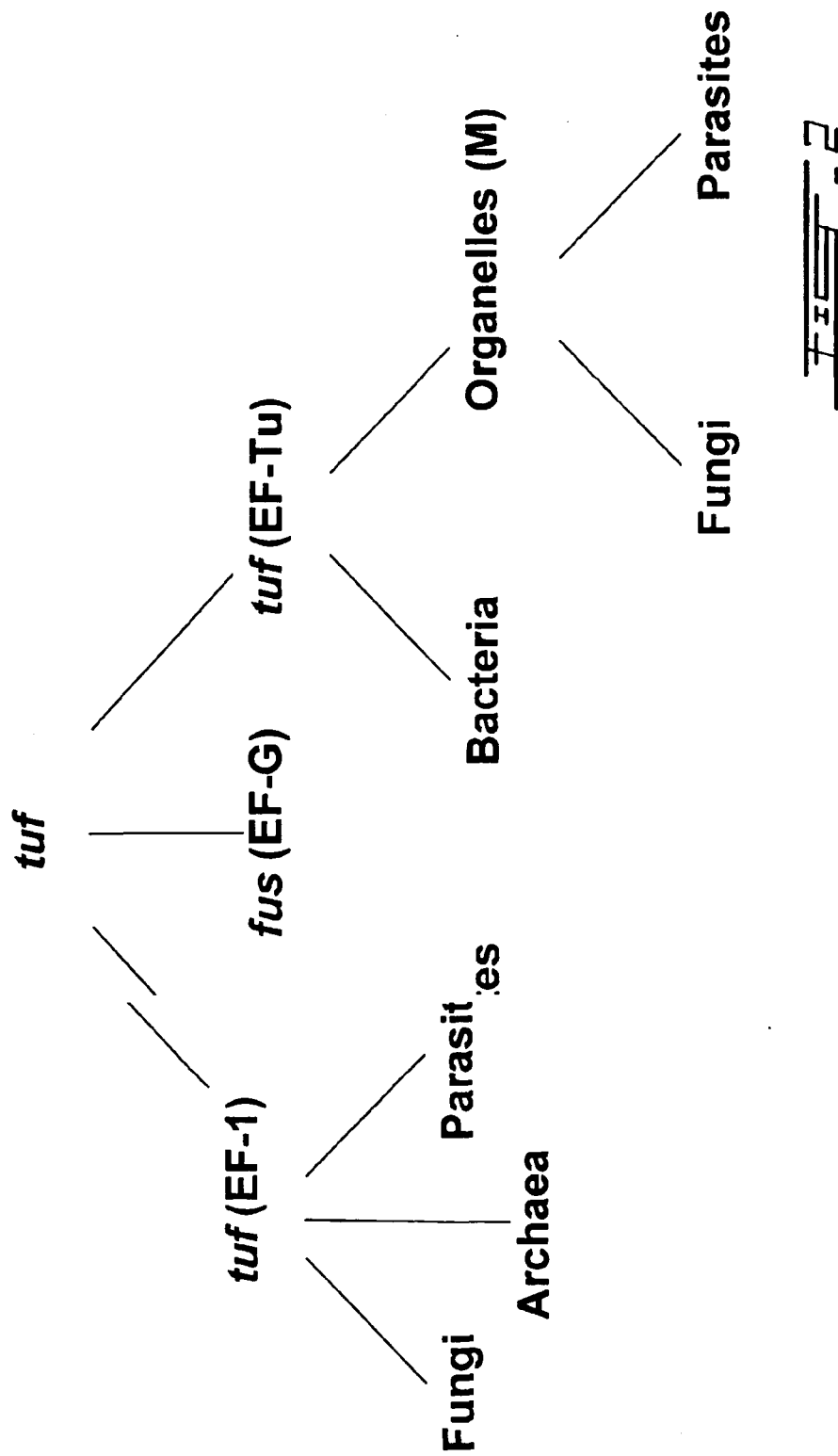
wherein said oligonucleotide probes and/or primers are capable of:

- a. specifically amplifying their target nucleic acids using PCR conditions comprising for each amplification cycle a denaturation step of 1 s at 95°C and an annealing-extension step of 30 s at 50-65°C ; or
- b. specifically hybridizing to their target nucleic acids under the following conditions:
 - hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:
 - aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and
 - bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

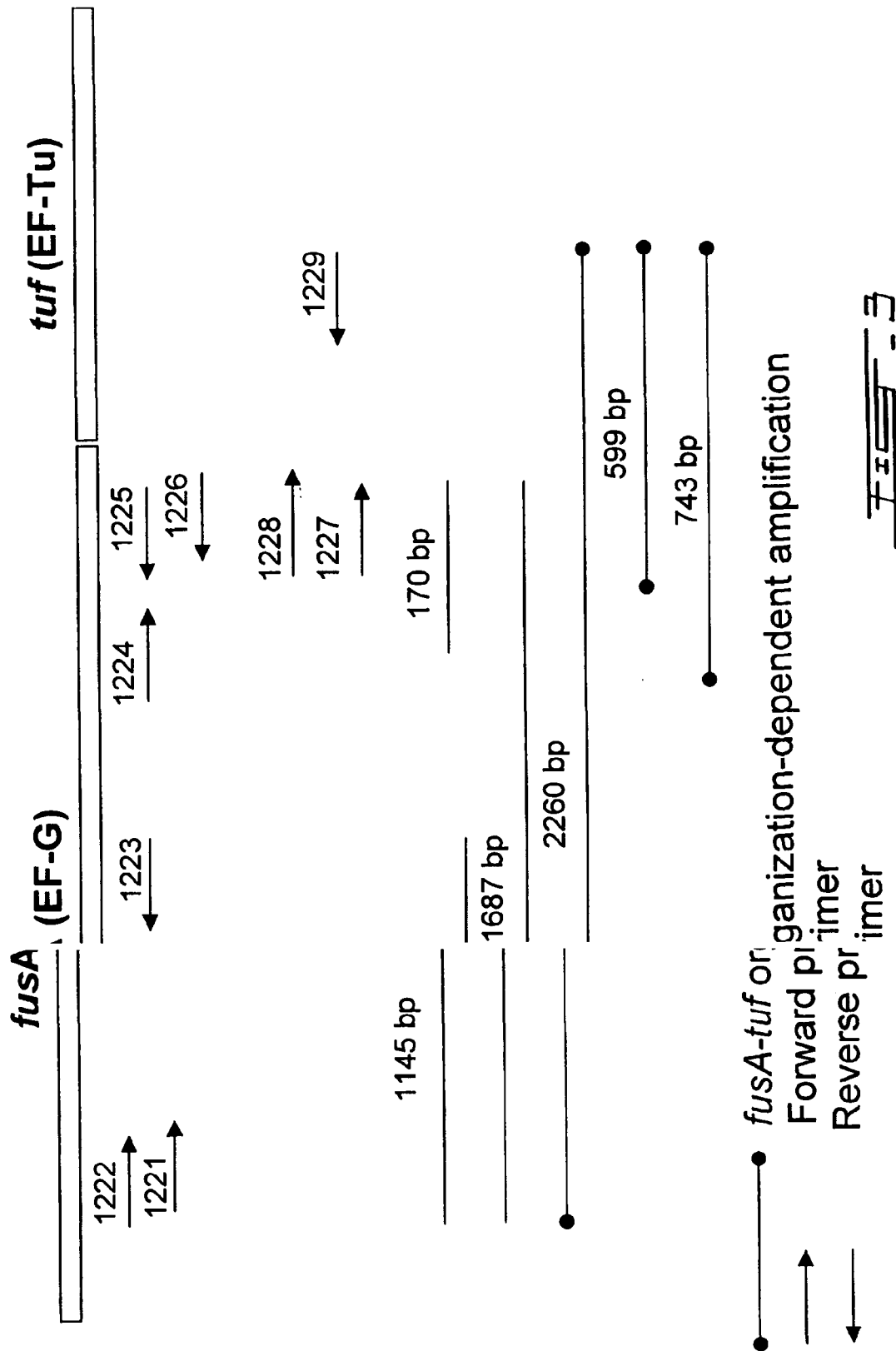
15. The diagnostic kit of claim 14, comprising the isolated *tuf* oligonucleotide of any one of claims 11-13.



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<i>S. aureus</i>	TPERDSKPFMMPVEDVI	220	230	240	250	260	270	280	290
<i>S. epidermidis</i>	TPERDSKPFMMPVEDVI	:SITGRGTIVATGRVERGQIKVGEEVEIIGLH.DTSKTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDVQRGVLA							
<i>S. durans</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQIKVGEEVEIIGMH.ETSKTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDVQRGVLA							
<i>E. hirae</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGVDWDIVGIAEETAQTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. mundtii</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGVDWDIVGIAEETAQTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. faecium</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGVDWDIVGIAEETAQTTVTGVMFMRKLLDYAEAGDNIGALLRGVSREDIQRGVLA							
<i>E. cecorum</i>	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEVVGIAEETSKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. columbae</i>	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIHDEISKTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. cassel. flavus</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIADETSKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. gallinarum</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIAEETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. faecalis</i>	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIADETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. avium</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIDETSKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. raffinosus</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIADETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. dispar</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIAEETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. malodoratus</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIAEETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLS							
<i>E. pseudoavium</i> (A)	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIAEETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. sulfureus</i>	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGDEVEIVGIAEETAKTITVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. saccharolyticus</i>	TPERDNDKPFMMPVEDVI	:SITGRGTIVATGRVERGQVRVGVDWDIVGIADETAQTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
<i>E. solitarius</i>	TPERDNDKPFMMPIEDVI	:SITGRGTIVATGRVERGQVRVGDWVEIVGIDEEETAQTTVTGVMFMRKLLDYAEAGDNIGALLRGVAREDIQRGVLA							
	FSITGRGTIVATGRVERGQIKVGDEVDIIGIHEDVKKTTVTGVMFMRKLLDYAEAGDNIGTLLRGVSRDDIERGVLA								

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<i>L. monocytogenes</i>	REHLLSRQVGVPYIIV	FMKCDIVDDEELLEVEMEIRDLLTEYEFPGDDIPVIGSALKALQ		CEADWEAKIDELMEAVDSYIP
<i>E. cassel. flavus (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLTEYDFPGDDIPVIGSALKALE		GDPDAEAAITLMDTVDEYIP
<i>E. gallinarum (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDSYIP
<i>E. durans (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDSYIP
<i>E. faecium (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALQ		GDPDAEAAIMELMDTVDEYIP
<i>E. hirae (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALQ		GDPDAEAAIMELMDTVDEYIP
<i>E. mundtii (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALQ		GDPDAEAAIMELMDTVDEYIP
<i>E. avium (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALQ		GDPDAEAAIMELMDTVDEYIP
<i>E. pseudoavium (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>E. malodoratus (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>E. raffinosus (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>E. dispar (B)</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>S. pneumoniae</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>S. suis</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>S. pyogenes</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>S. mutans</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDEYIP
<i>L. lactis</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDSYIP
<i>T. aquaticus</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDSYIP
<i>E. coli</i>	REHLLSRQVGKHLIV	FMKCDIVDDEELIDVEMEVRELLSEYDFPGDDIPVIGSALKALE		GDPDAEAAIMELMDTVDSYIP

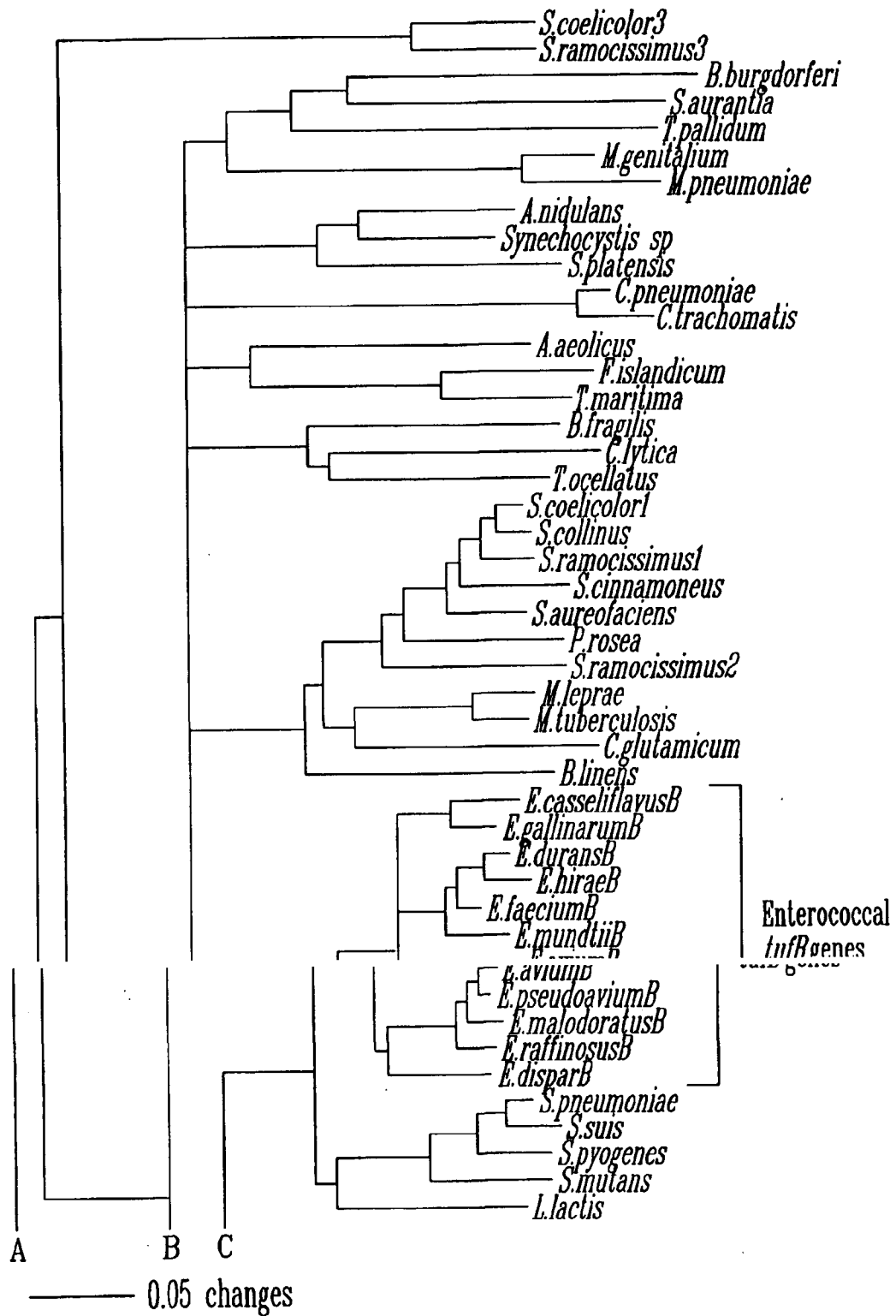
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$$\begin{array}{ccccccc} \text{a2} & \text{b2} & \text{c2} & \text{d2} & \text{e2} & \text{f2} & \text{g2} \\ \text{h2} & \text{i2} & \text{j2} & \text{k2} & \text{l2} & \text{m2} & \text{n2} \end{array}$$

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L. monocytogenes (B)
E. cassel. flavus (B)
E. gallinarum (B)
E. durans (B)
E. faecium (B)
E. hirae (B)
E. mundtii (B)
E. avium (B)
E. pseudoavium (B)
E. malodoratus (B)
E. raffinosus (B)
E. dispar (B)
S. pneumoniae
S. suis
S. pyogenes
S. mutans
L. lactis
T. aquaticus
E. coli

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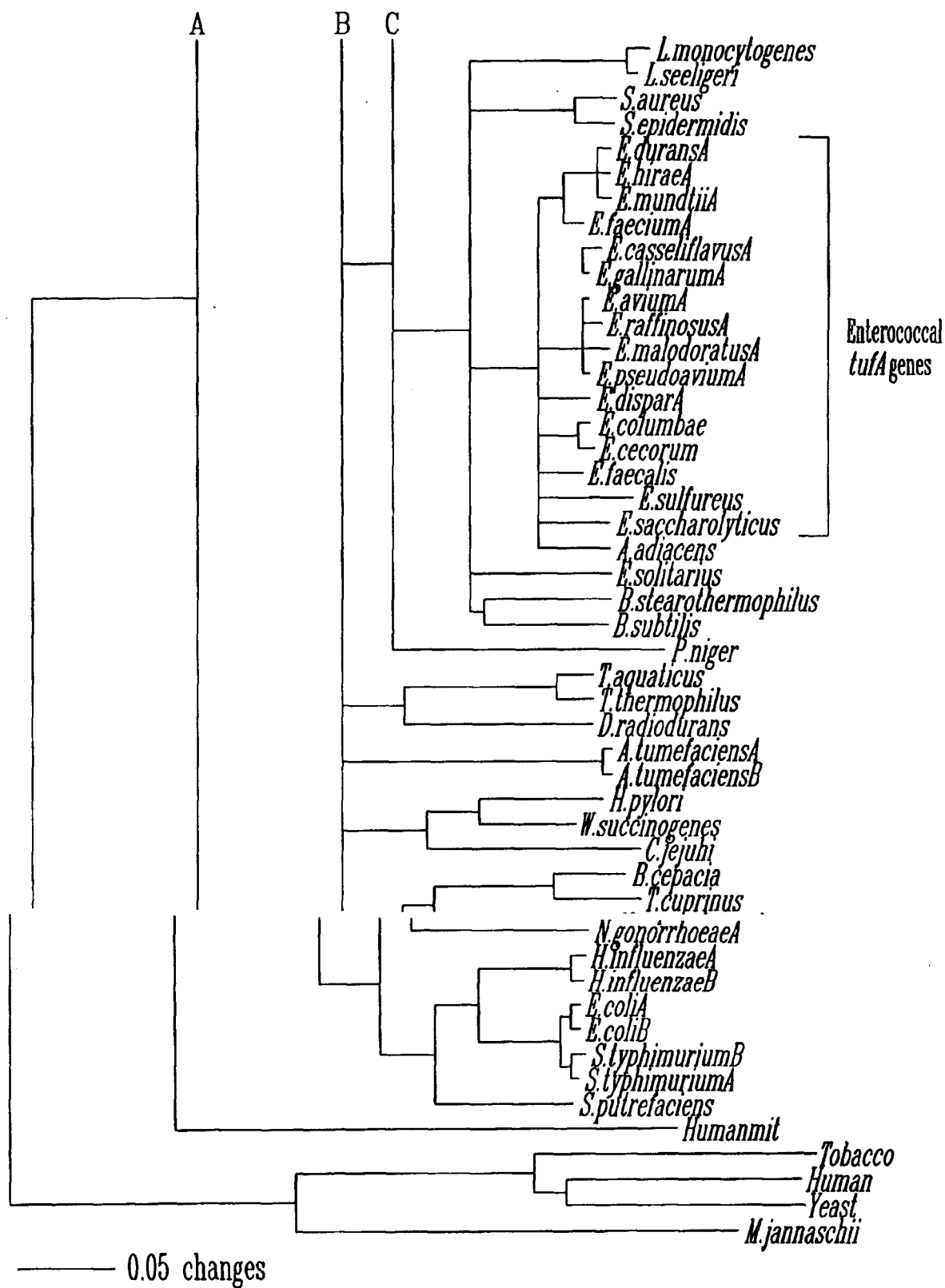
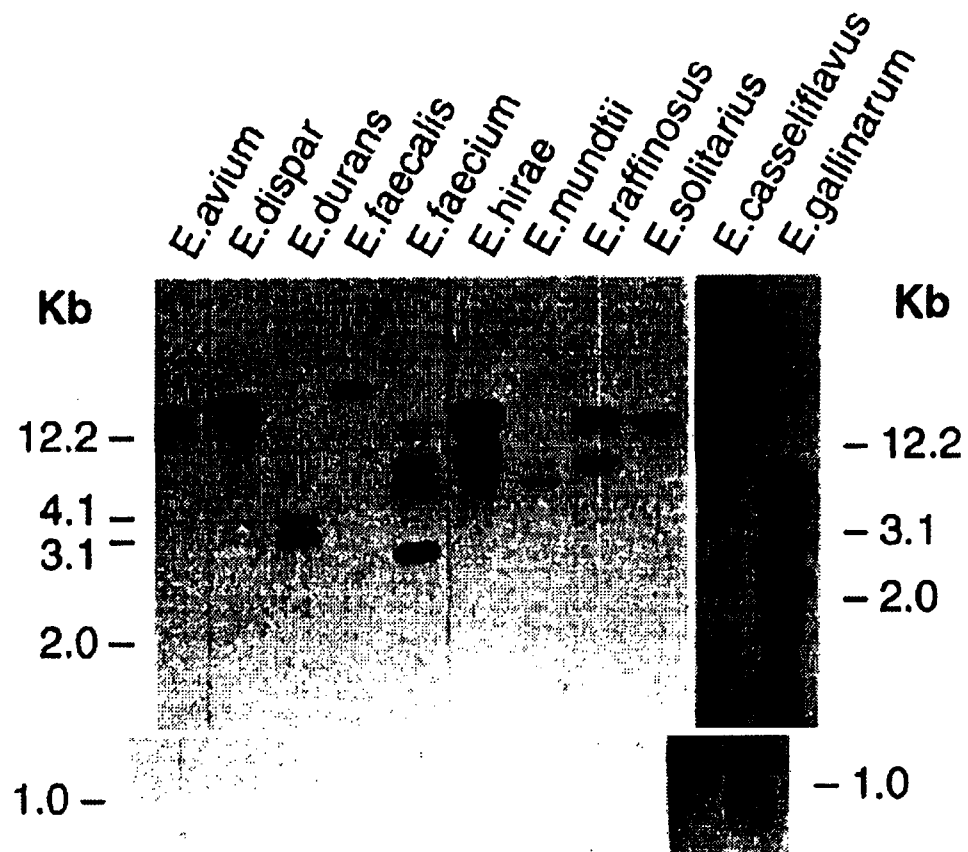


FIG. 5b

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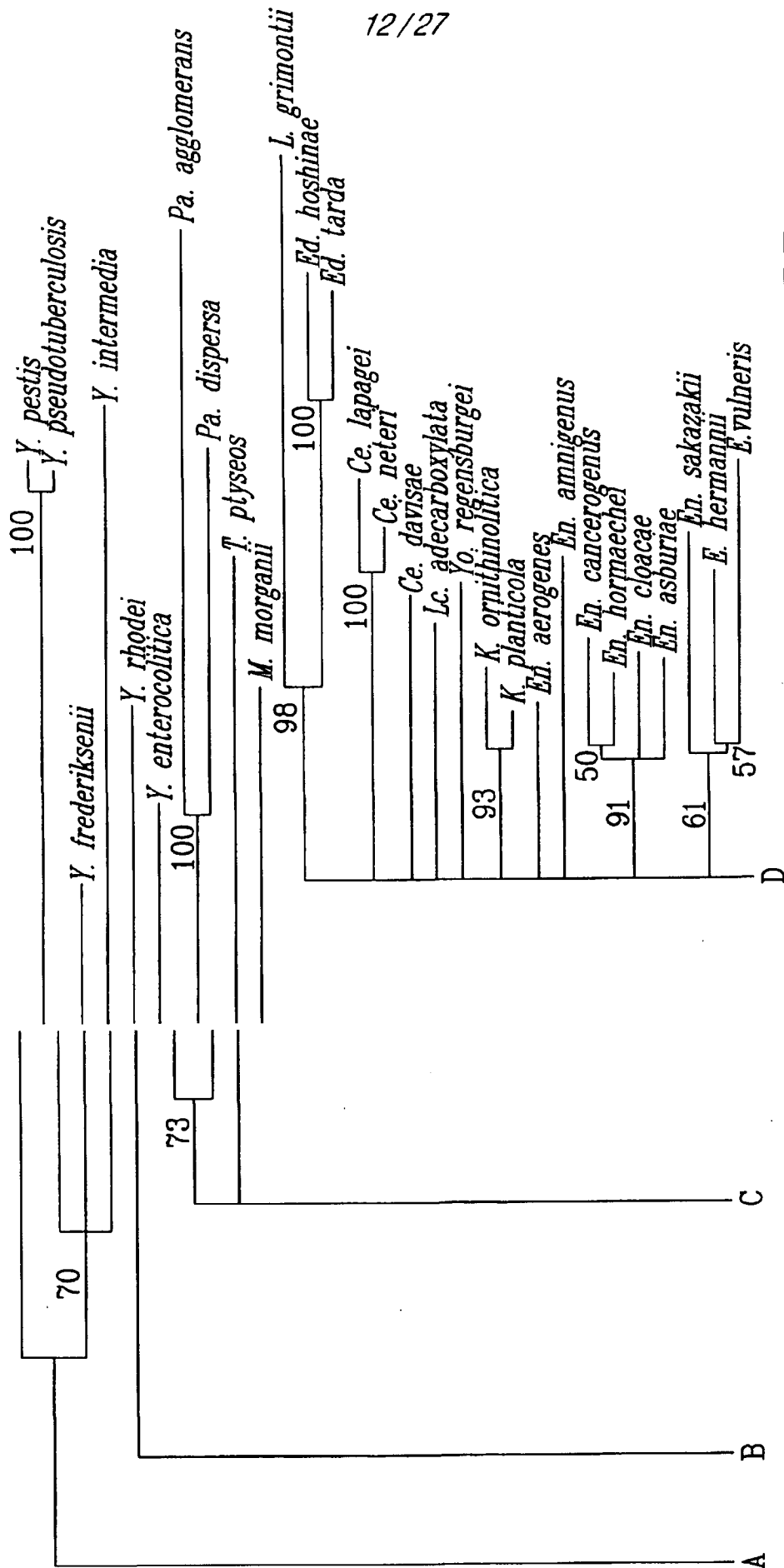


T E S - 6

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301	311	321	331
E. coli	GAGATCGGT	AAGAAGAGCG TTGGG	CGATTCACCG
E. agglomerans	GACATCGGT	AAGAAGAGCG TTGGG	CGATCCACCG
P. agglomerans	GAGTGAAA	AAGAAGATGG CAGCGCAGTA GAGATCGCCT	CTATTCACCG
P. dispersa	GACCTGAAA	AAGAAGACGG CAGCGCTGTA GAGGTTTCCT	CTATTCATCG
T. ptyseos	GACCTGAAG	ACGAAGATGG TAGCAATGTT GAGGTGAAC	CTATTCACCG
E. coli	~I~G~E~	~E~E~R~W~A~	I~H~R~
E. agglomerans	~I~G~E~	~E~E~R~W~A~	I~H~R~
P. agglomerans	~L~K~E~	~E~D~G~S~A~V~E~	I~H~R~
P. dispersa	~L~K~E~	~E~D~G~S~A~V~E~	I~H~R~
T. ptyseos	~L~K~N~	~E~D~G~S~A~V~E~	I~H~R~

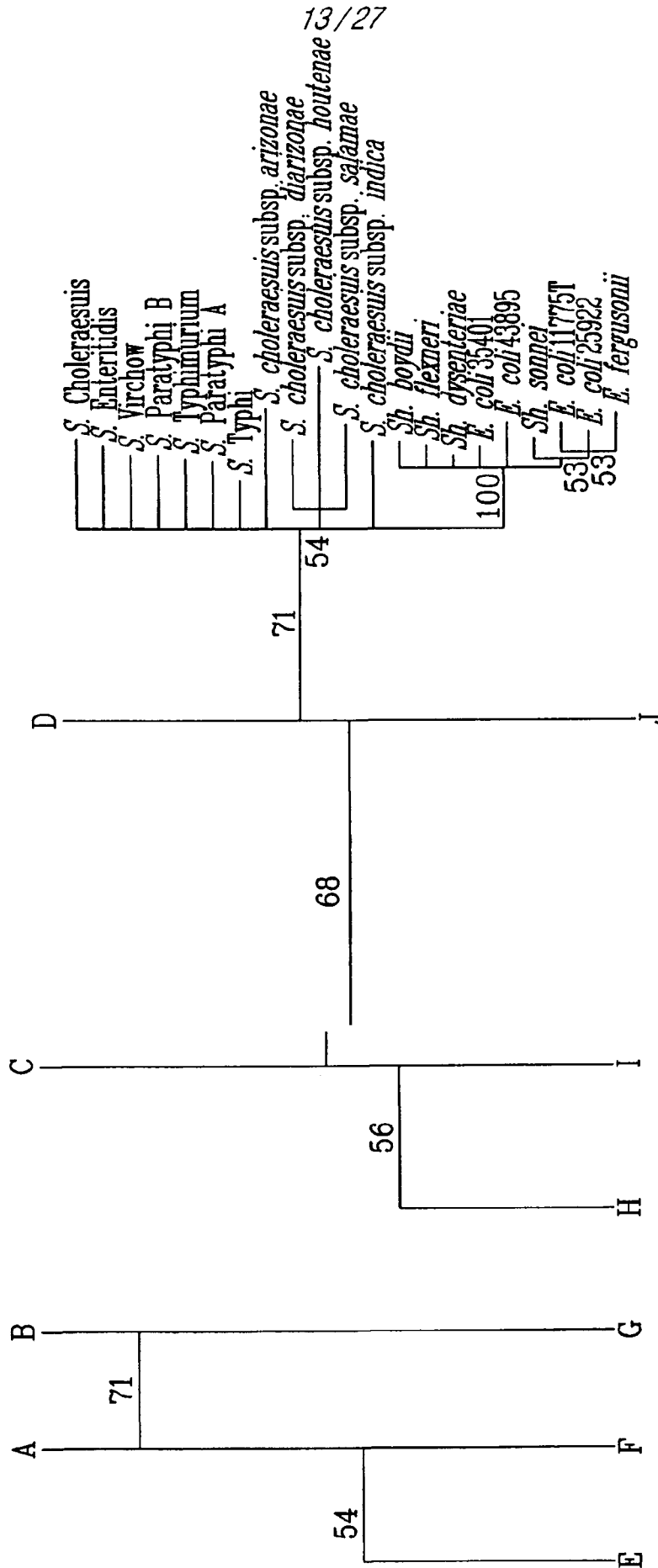
FIG - 7



711-123

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FE-86

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PCT/CA00/01150

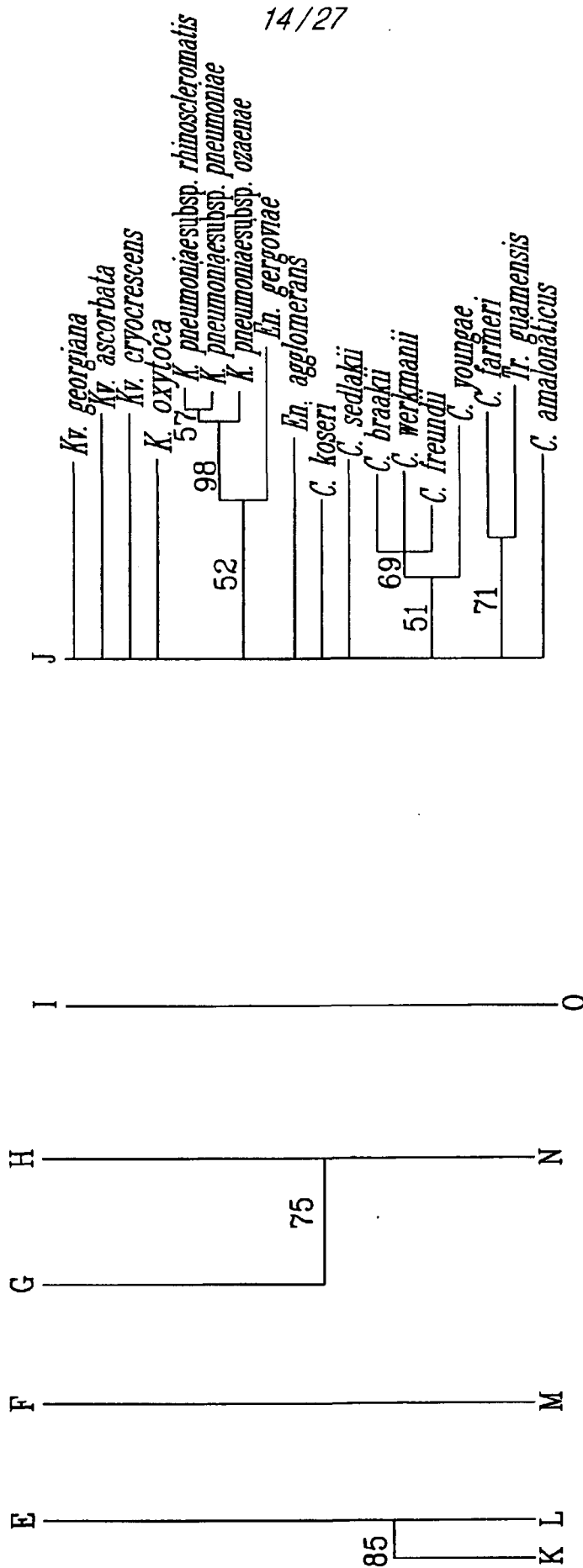
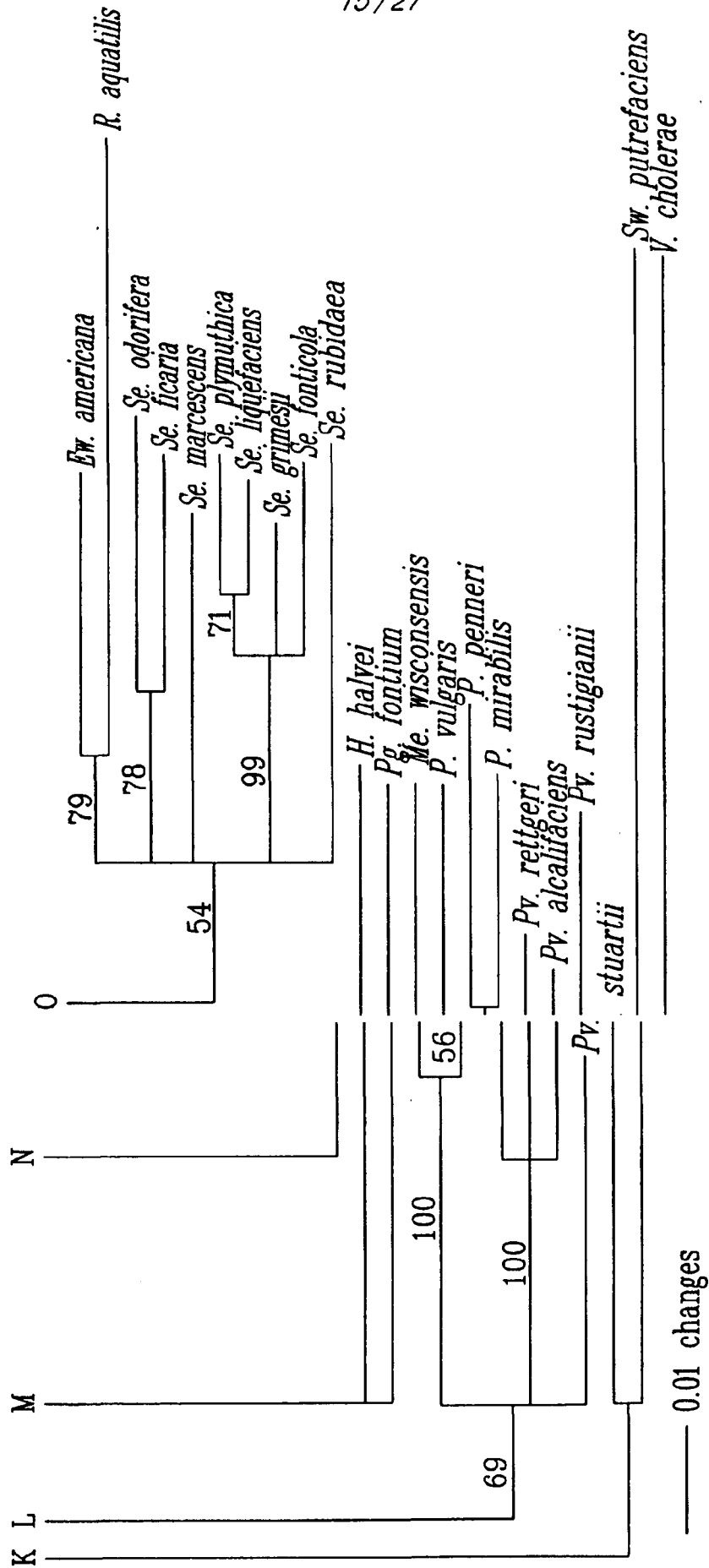


FIG. 1

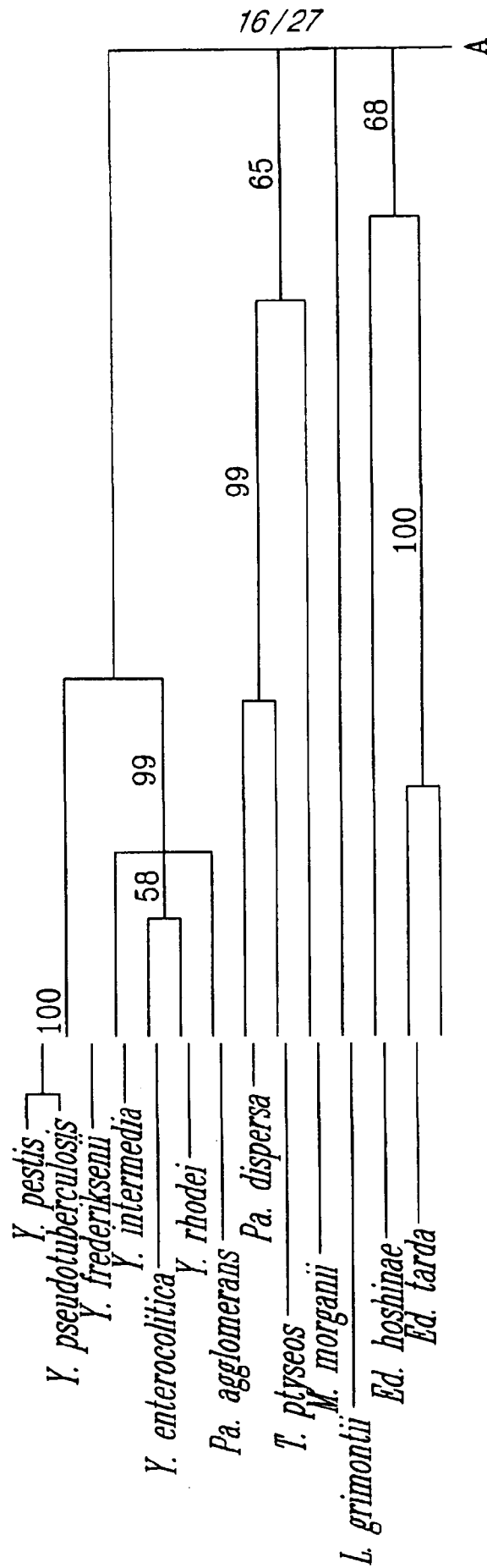
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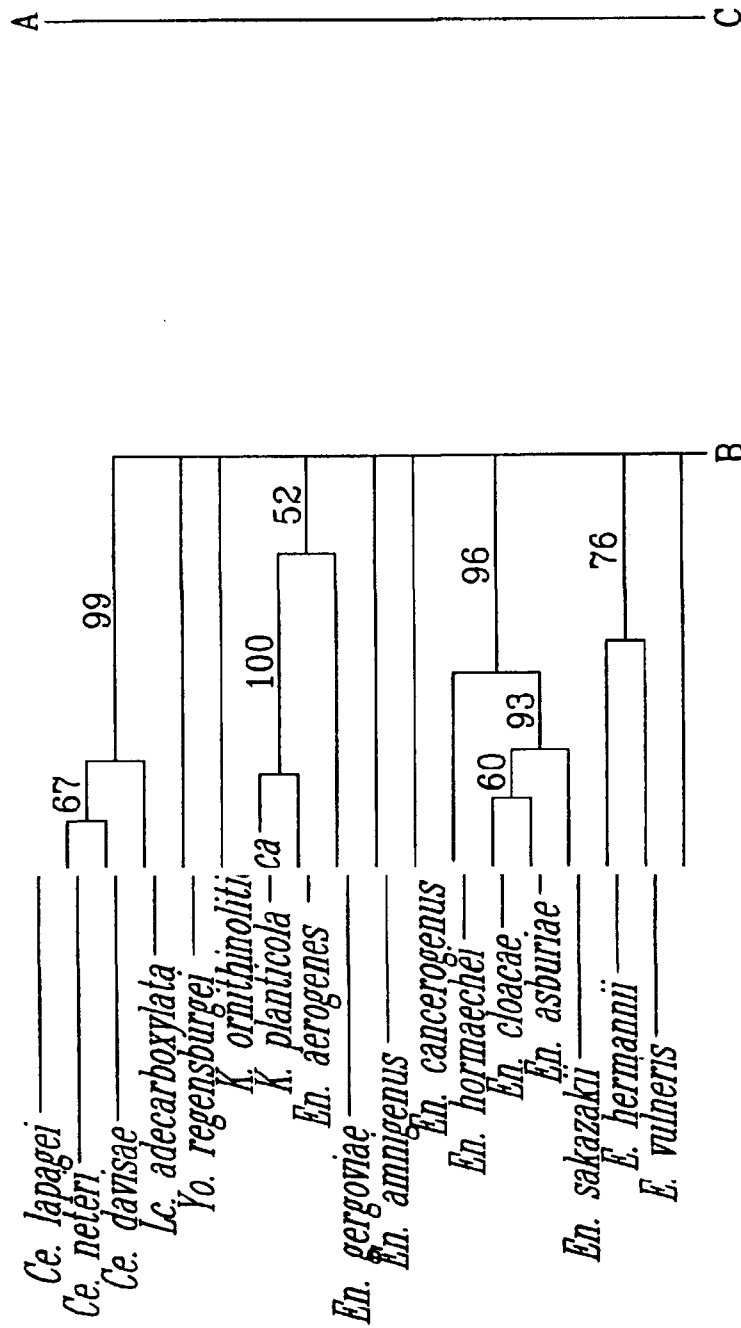
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PCT/CA00/01150



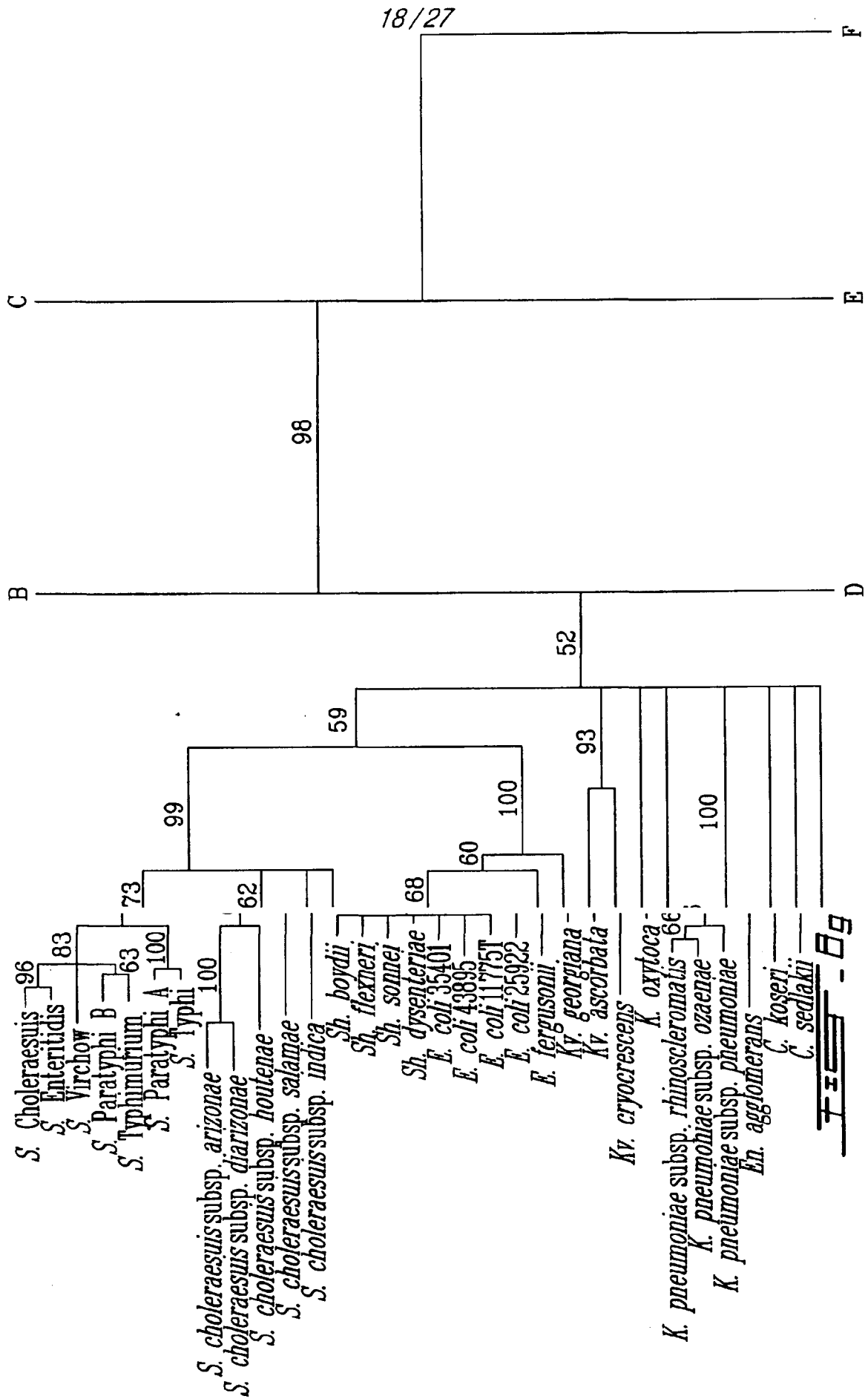
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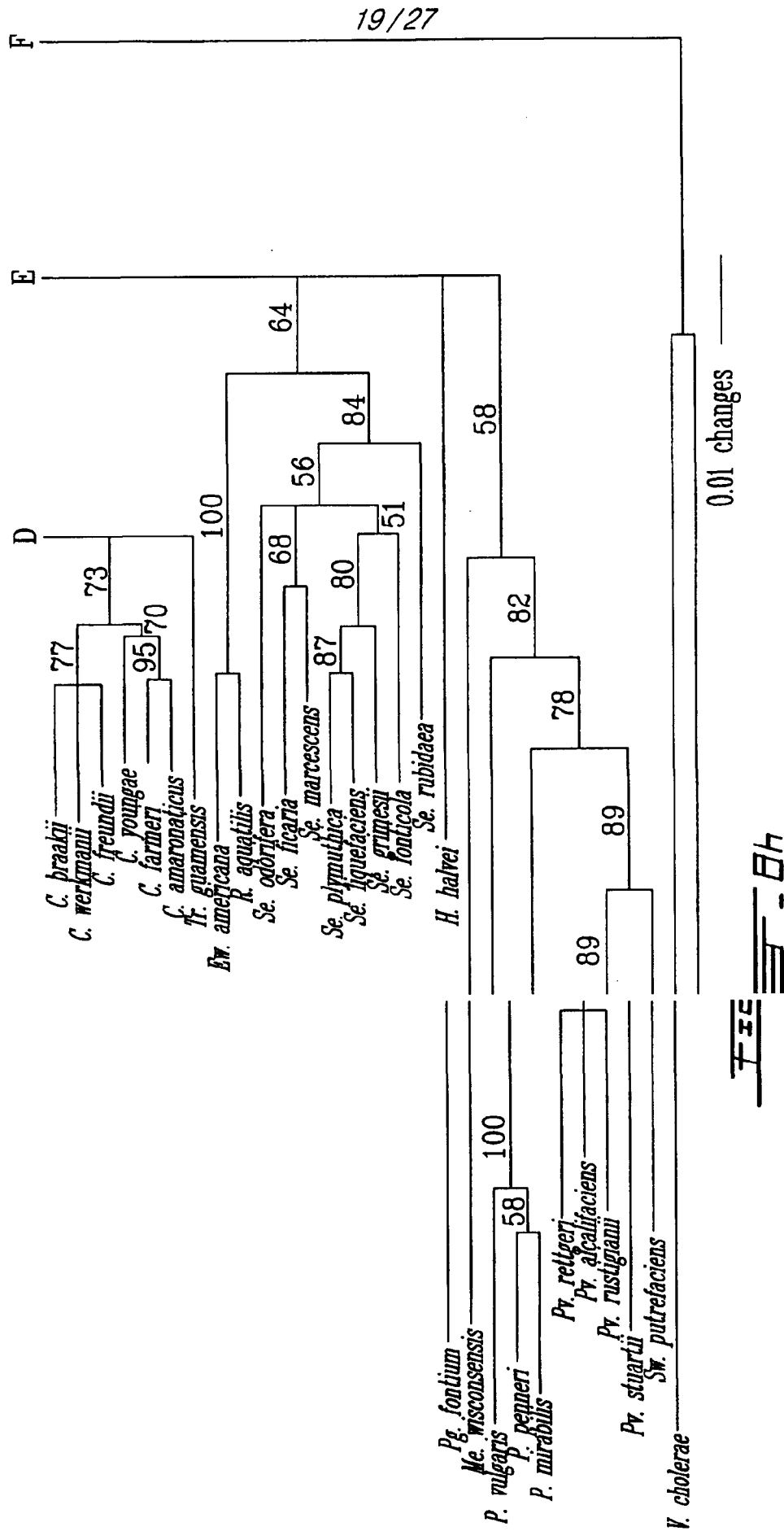
WO 01/23604

PCT/CA00/01150

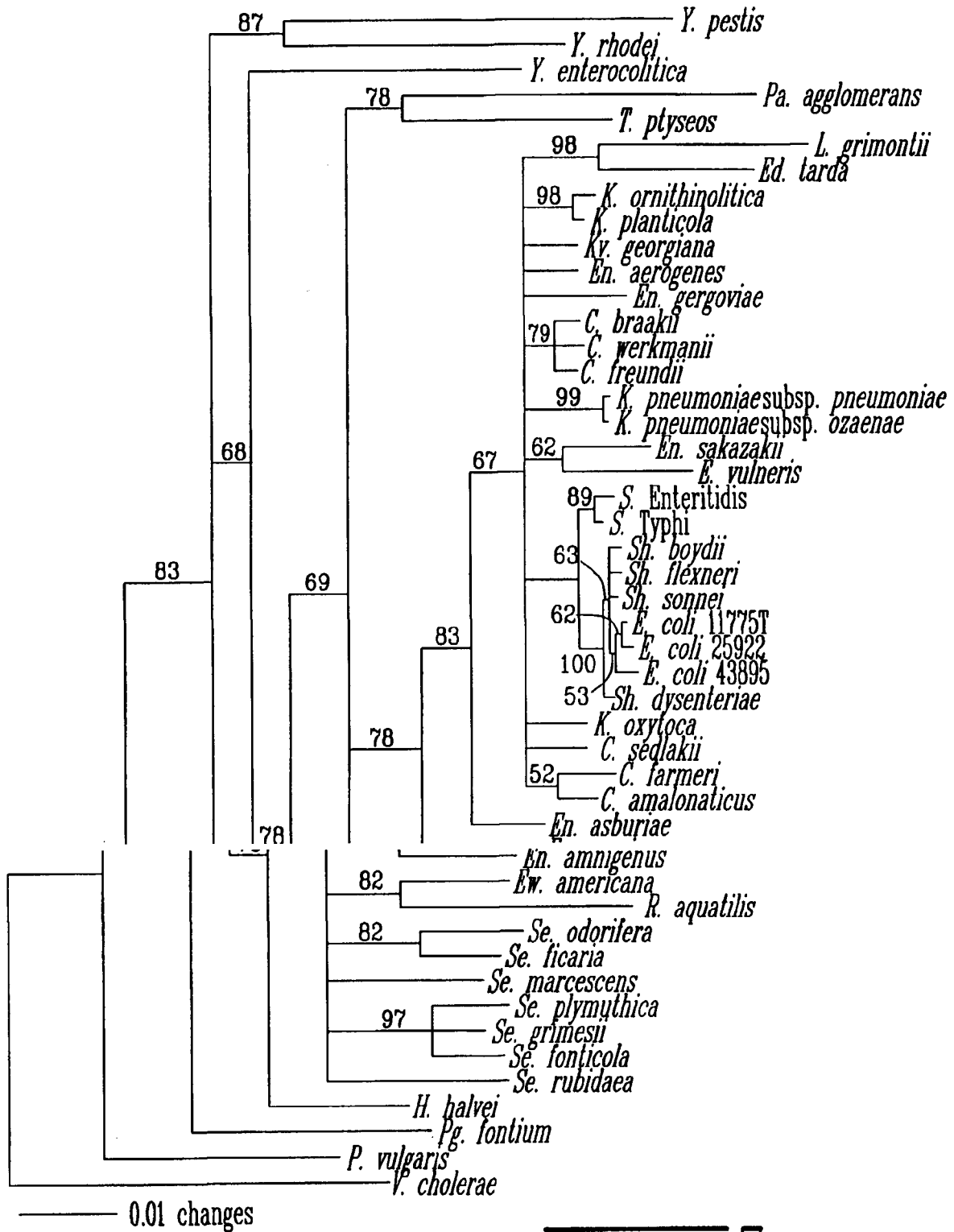


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PCT/CA00/01150

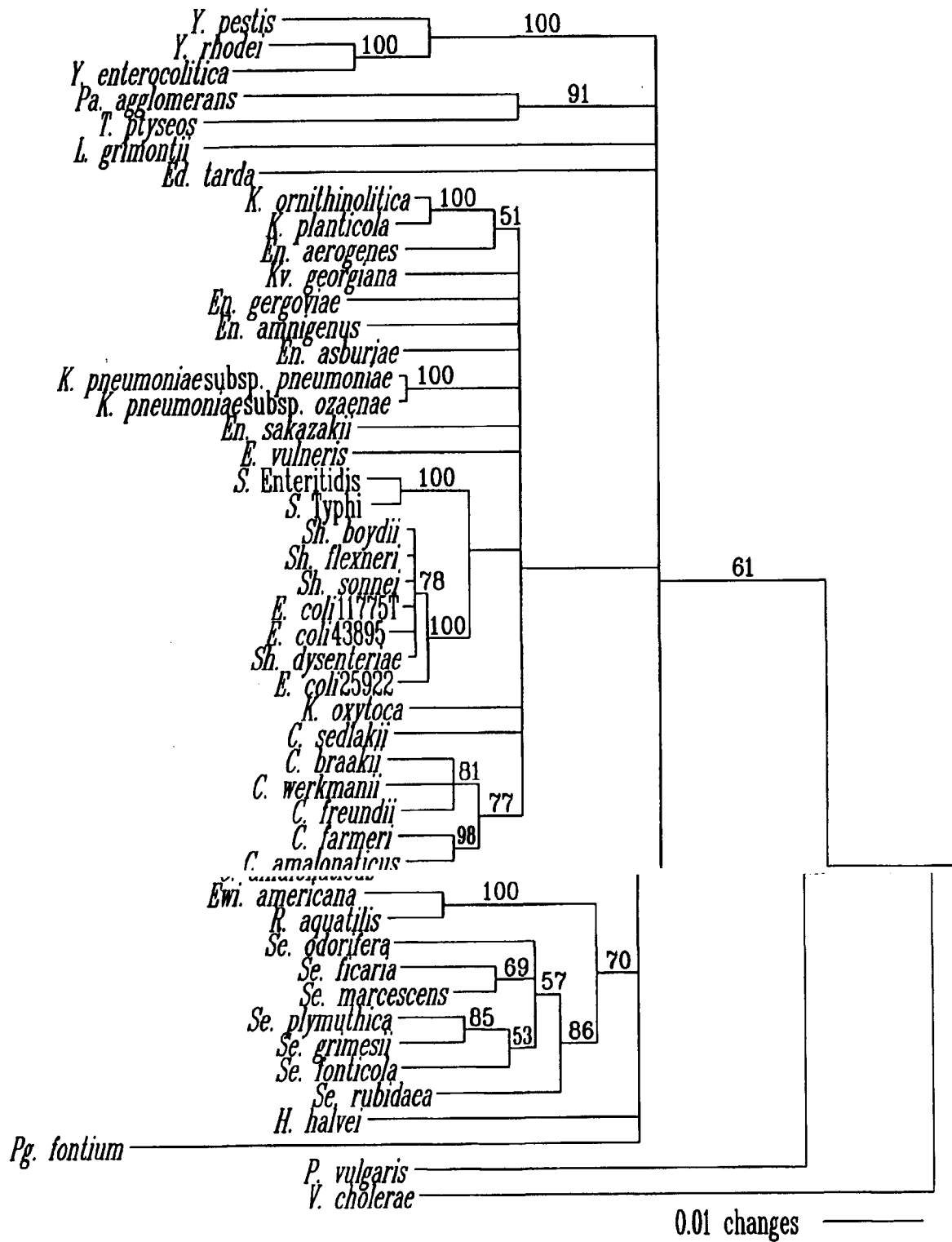


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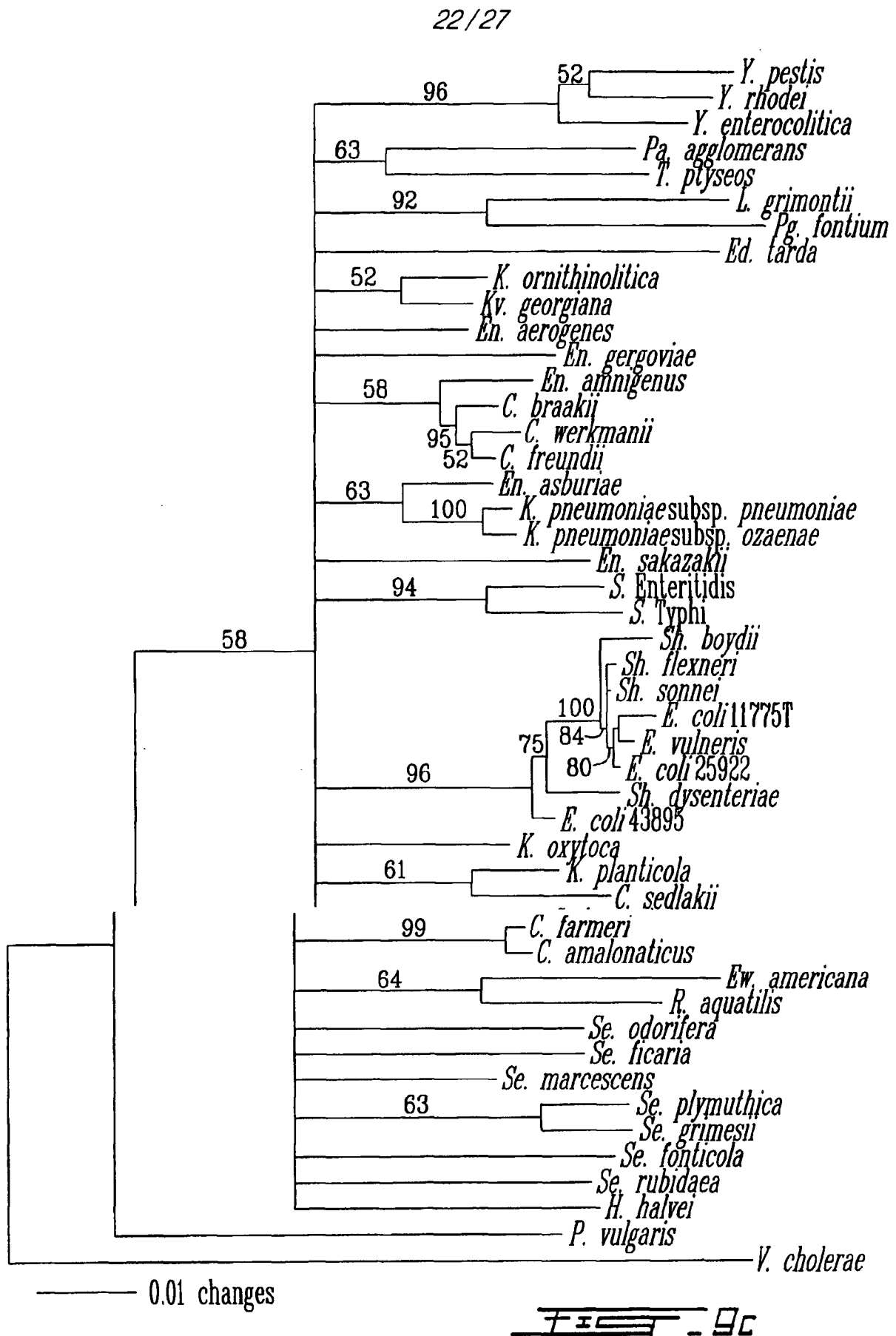


FIS - 9a

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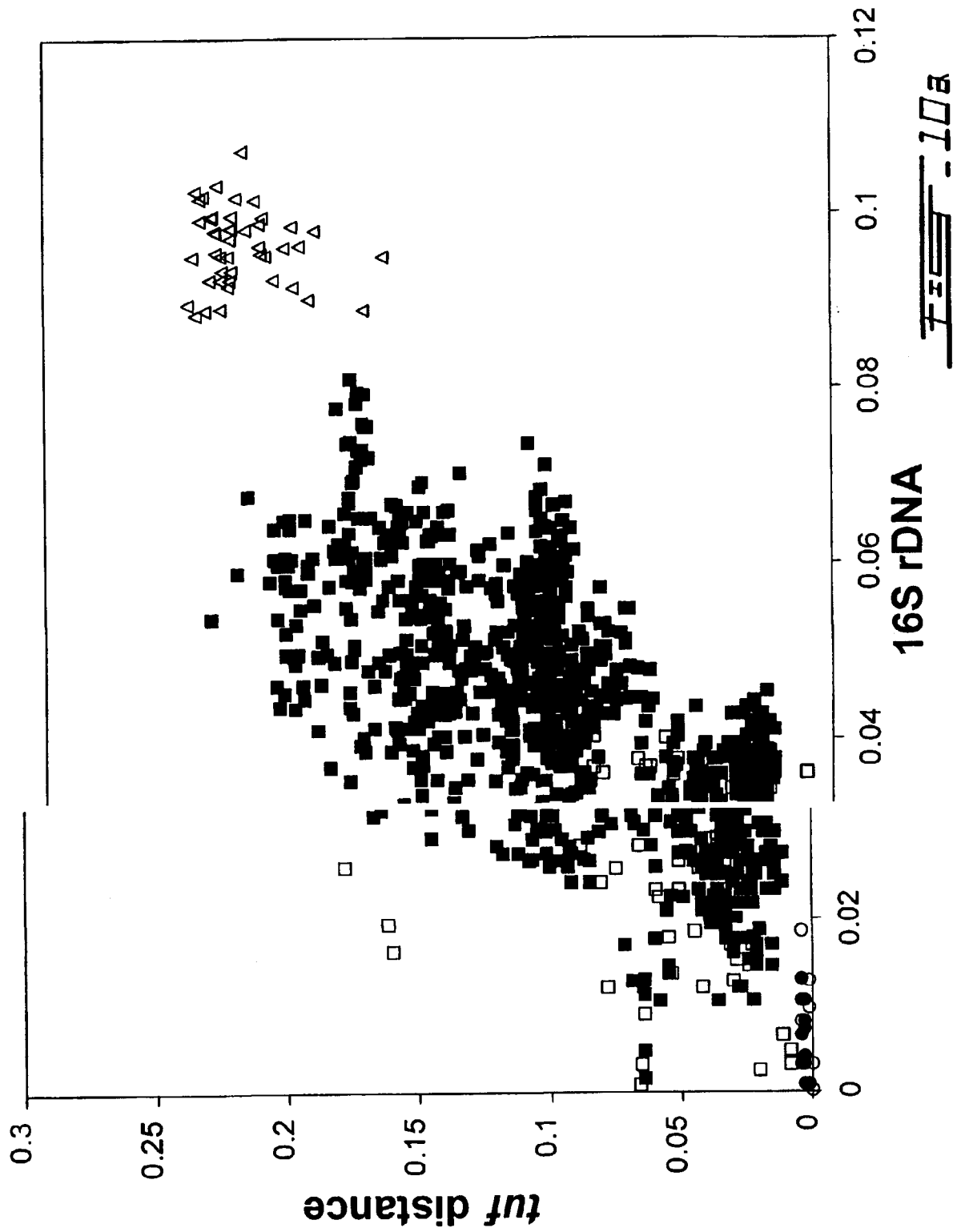
FIS-96



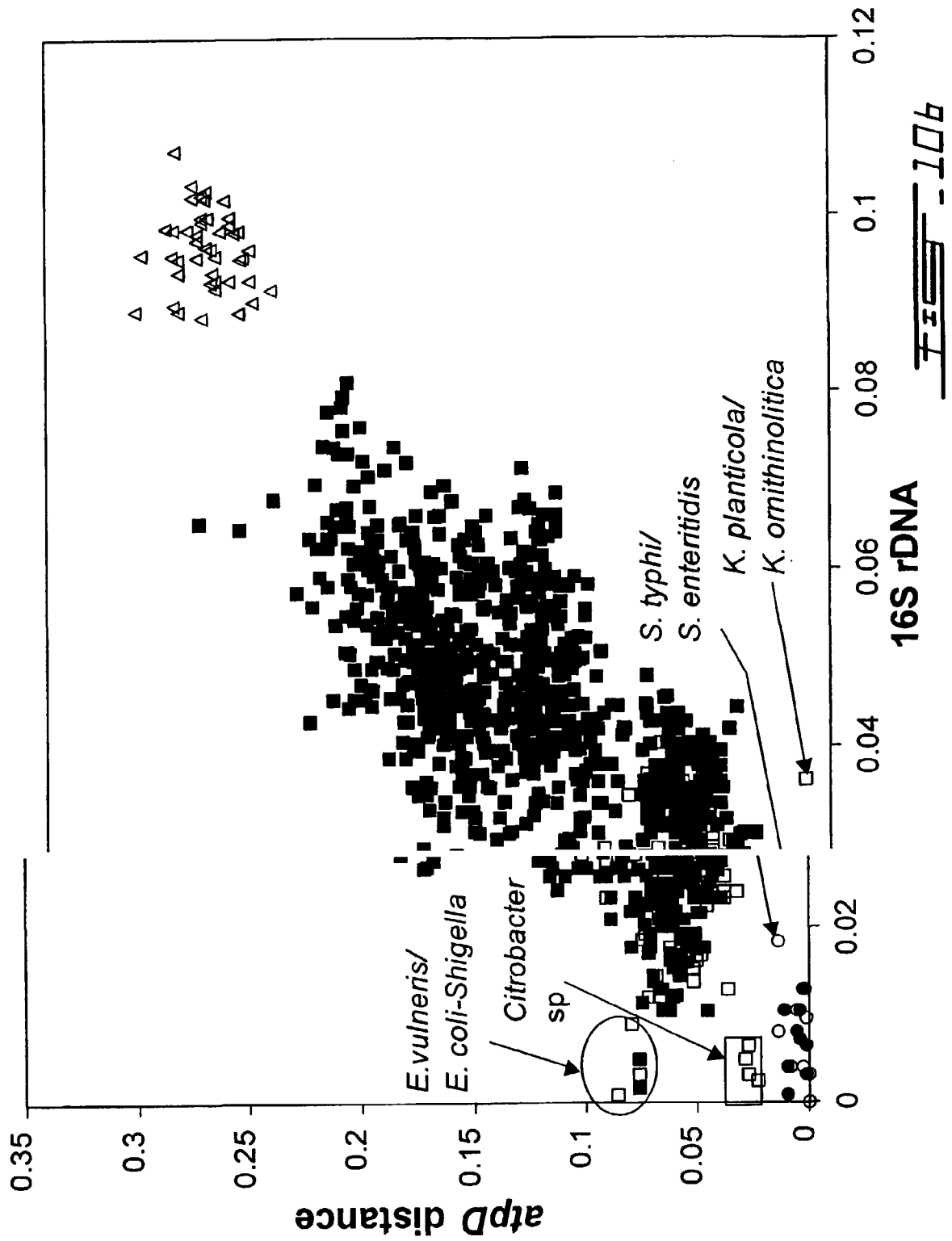
WO 01/23604

PCT/CA00/01150

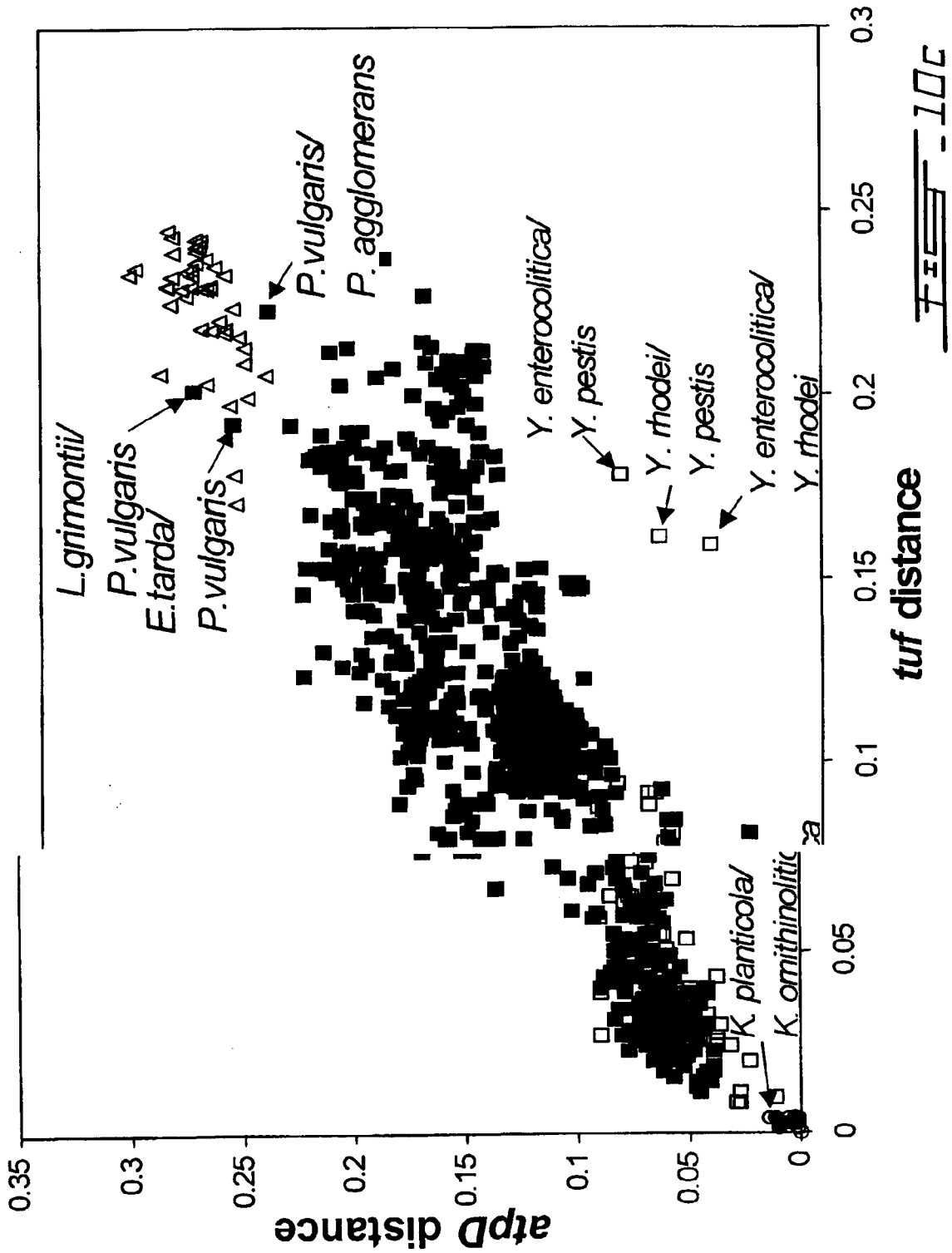
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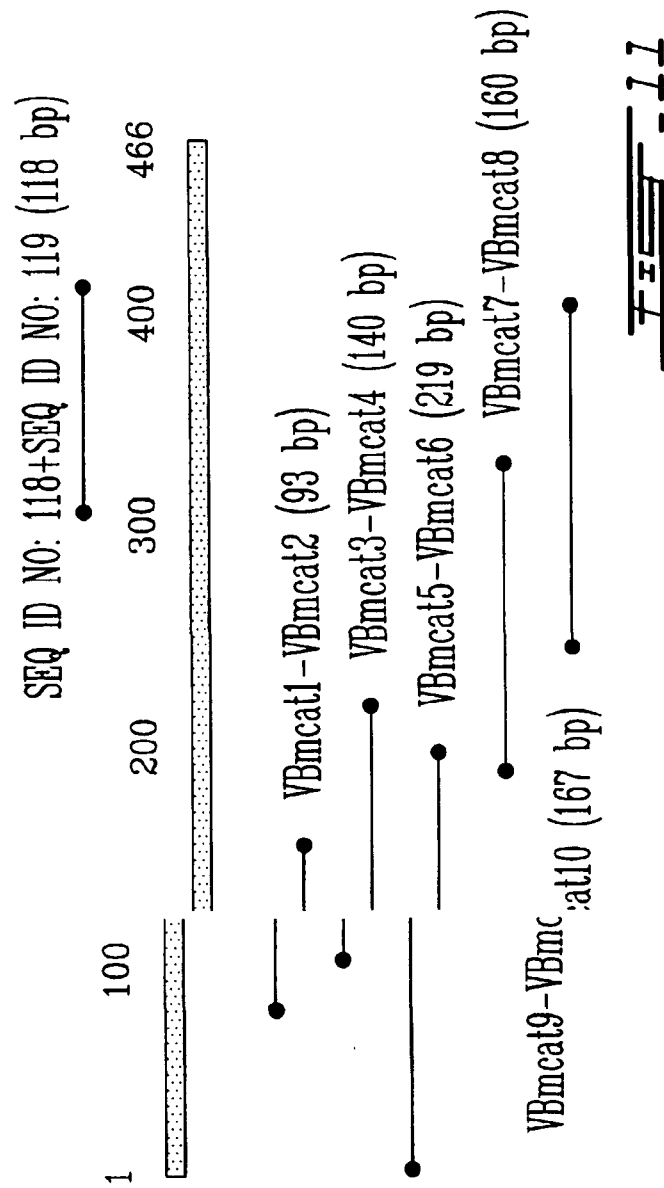
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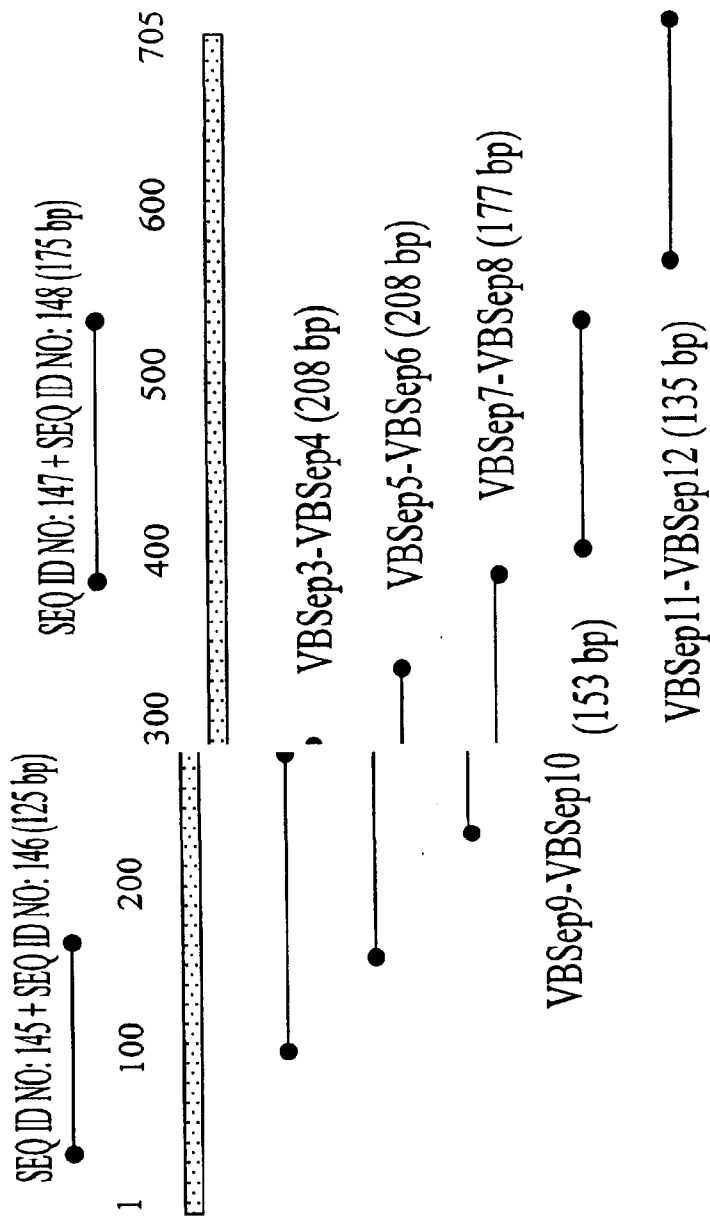


FIG. 12

atpD

***atpD* (F)**

***atpD* (V)**

Bacteria

Eukaryotes

Bacteria

Eukaryotes

Archaea

Fungi

Parasites

Fungi

Parasites

