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(54) Titre : SONDES POUR LE DIAGNOSTIC D'INFECTIONS DUES AU BACTEROIDES FRAGILIS
(54) Title: PROBES FOR THE DIAGNOSIS OF INFECTIONS CAUSED BY BACTEROIDES FRAGILIS

(57) Abrégé/Abstract:
The DNA from the bacteria Bacteroides fragilis is extracted, then completely digested with restriction enzyme HindIII, followed by cloning into a suitable vector to select a probe comprising DNA which is essentially contained in Bacteroides fragilis , then the sequence of the probe is elucidated.

ABSTRACT

The DNA from the bacteria *Bacteroides fragilis* is extracted, then completely digested with restriction enzyme HindIII, followed by cloning into a suitable vector to select a probe comprising DNA which is essentially contained in *Bacteroides fragilis* , then the sequence of the probe is elucidated.

5

SPECIFICATION

Probe for the Diagnosis of Infections Caused by *Bacteroides fragilis*

5

[Field of the Invention]

The present invention relates to a probe which is useful for detecting and identifying *Bacteroides fragilis*, the causative bacteria of infectious diseases such as intraperitoneal infection, female genital infection, sepsis and the like.

10

[Back Ground Art]

Generally, the diseases caused by infection of pathogenic microorganisms are called infectious diseases. In pathology, "infection" is defined as an invasion of pathogenic microorganisms (hereinafter referred to as "bacteria") and an
15 establishment of footholds for the growth in the host organism by the pathogenic microorganisms. Thereafter, the outbreak of the disease states caused by proliferation of the pathogenic microorganisms *in vivo* depends upon the relationship between the resistance of the host and the virulence of the bacteria.

Anaerobic bacteria are accounted for 20% of total bacteria strains isolated
20 from various kinds of infectious diseases. In particular, the gram-negative anaerobicbacteria reach to 30 to 50% of the total anaerobic bacteria, and *Bacteroides fragilis* is known to be the most frequently detected strain among them.

In Bacteriology, *Bacteroides fragilis* is taxonomically classified as a nonsporing anaerobic gram-negative bacterium, which is resident within human digestive tract,
25 external genitalia, vagina and urethra. Particularly in colon, it is present at 10^9 to 10^{11} per gram of feces, and the number thereof is even higher than that of *Escherichia coli.*

Bacterioides fragilis is the causative bacteria of the endogenous infection of which pathological role is opportunistic. Clinically, this bacteria is detected at the
30 higher rate in intraperitoneal infectious diseases, female genitoinfectious diseases, decubitus, diabetic ulcer, osteromyelitis, bacteremia, the infections in soft tissues of lower half of the body and the pus therefrom (*Chemotherapy*, Vol. 37, pp1229-1244,

(1989); *Rinsho Kagaku*, Vol. 22, pp322-333 (1986)). Furthermore, intraperitoneal abscess is elicited as a result of the involvement of the bacteria when the perforation is caused by trauma in intestinal tracts, especially in colon, surgical procedure, ulcer, cancer, diverticulitis or appendicitis. The presence of anaerobic bacteria has been
5 demonstrated in 90% of the cases of the intrapelvic abscess, and approximately the half of these cases may be related to *Bacterioides fragilis*. Additionally, the anaerobic bacteria are the pathogens for 5 to 10% of bacteremia, and the involvement of *Bacterioides fragilis* in these symptoms has been also known.

Nonsporing anaerobic bacteria such as *Bacteroides fragilis* are generally
10 resistant to the antibiotics of aminoglycosides and polymyxin derivatives. Particularly, since *Bacteroides fragilis* produces β-lactamase, a tolerance for many of β-lactam agents such as penicillin and cepham have been gained. Also, a remarkable resistance against ordinarily administered antibacterial agents have been imparted, in comparison with the other anaerobic bacteria. The cephamycin-resistant
15 and imipenem-resistant lines have been discovered as well (see, "*Kagaku Ryoho no Ryoiki*", Vol. 6, No. 9, 1915-1925 (1990)). Therefore, it is extremely difficult to treat the patient, once the infection by such bacteria is established. The higher mortality has been reported, particularly in the cases that the infection lead to sepsis. Additionally, the infection of these bacteria may rather be drawn as "replacement of
20 bacteria" through pre-administration of antibiotics before the surgical procedures.

Thus, there is a continuous need for the establishment of the method for rapid diagnosis of the infectious disease caused by *Bacteroides fragilis*, since the accurate diagnosis at an early stage of the infection and the selection of the appropriate agents are extremely crucial for the therapeutic treatment.

25 Infections caused by anaerobic bacteria are generally initiated by the invasion of indigenous bacteria into the tissue through a local rupture.

In the conventional diagnostic procedure, it is mandatory to : (1) analyze the clinical symptoms; (2) culture the specimen; and (3) Gram stain the bacteria which are found in the specimen, and then the therapeutic strategy is determined after these
30 items are sufficiently examined. Actually, the following findings may tentatively suggest the suspected *Bacteroides fragilis* infection:(1) gram-negative bacteria in the specimen detected by direct Gram stain; (2) foul-smelling specimen; (3) infections

found in the parts under diaphragm; (4) infectious diseases which are not effectively alleviated by any treatment using penicillin or cephalosporin derivative agent; (5) the patient suffering from diabetic ulcer, decubitus, or bacteremia. However, it is necessary to search for the correct bacteria which caused the infection, then the
5 bacteria must be identified in order to attain the accurate diagnosis. Thereafter, the appropriate antibiotics adequate for the treatment of thus identified bacteria should be administered. Therefore, the rapid and accurate method to identify the causative bacteria has been desired in the clinical field.

In addition, the identification of the causative bacteria has been increasingly
10 important in recent years, because of the discoveries of the several drug resistant causative bacteria.

However, the identification of the causative bacteria generally involves certain difficulties in actual clinical cases. In particular when the causative bacteria are anaerobic ones, every effort should be made in order to avoid the contamination of
15 indigenous bacteria, and the exposure of the bacteria to air and the dryness. In more detail, identification of the causative bacteria in the specimen from the patient suspected as an anaerobic bacteria infection, the specimen such as blood, spinal fluid, pleural fluid, ascites, puncture fluid from abscess, or samples from pus or secreted materials is collected and subjected to the analysis. Since the causative bacteria are
20 known as indigenous bacteria in human lower intestine and vagina, the specimen has to be obtained most carefully for avoiding the contamination of the indigenous bacteria, especially in the cases of the infections observed in these parts. Once the specimen is collected from the patient, it must be kept in the container designed suitable for anaerobic bacteria analysis, which immediately protects the specimen
25 from dryness and the contact with oxygen. In general procedure to identify the causative bacteria, thus obtained specimen is observed macroscopically and examined on the presence of odor, then followed by direct Gram stain on smear, and culture in the selected medium under an aerobic condition for 48 hours. Whereas, according to this procedure, a long proliferation period of the bacteria from the specimen, and
30 further, 3 to 4 days of incubation period would be required to attain the result of the drug sensitivity test. In addition, the cases in which *Bacteroides fragilis* can be detected as independently existing causative bacterial species are relatively rare, and

70 to 80 % of the cases in which *Bacteroides fragilis* was detected were reported to be the combined cases caused by plural kinds of bacteria. In some cases, the existing *Bacteroides fragilis* may not be detected because the specimen is treated aerobically when the detection of the aerobic bacteria is intended. Additionally, in cases of the diagnosis of the patients who had already been treated with a large dose of antibiotics when the possible infection was suspected, the growth and proliferation of the bacteria may be prevented even if the bacteria are present in the specimen. Accordingly, the feasibility of successful culture of the bacteria from these specimen may become extremely low.

Furthermore, alternative subroutine methods developed heretofore may include: an instrumental analysis method of constituents of bacteria and metabolic products from bacteria (See Yoshimi Benno, "Quick identification of bacteria with gas chromatography", *Rinsho Kensa*, Vol. 29, No.12 pp.1618-1623, November 1985, Igaku Shoin.); a method utilizing a specific antibody (See Japanese Patent Provisional Publication No.60-224068.); and a hybridization method utilizing a specificity of DNA (Japanese Patent Provisional Publication No. 61-502376), however, any of which requires the steps for isolation of the bacteria, as well as the steps for culturing and growing the bacteria.

On the other hand, an established method based on the function of the phagocyte in the infectious diseases has been proposed, wherein a stained smear of buffy coat in which leukocytes constituents in the blood sample are concentrated is examined under an optical microscope. Generally speaking, the detection rate of bacteria in buffy coat specimens from adult bacteremia patients is 30% at most, which is similar to that in blood specimens from ear lobes, however, it was reported that in case that the patients are newborn children, the bacteria could be detected in seven cases in ten (70%). Therefore, information concerning the presence of bacteria in peripheral blood obtained by a microscopic prospection on a smear can provide an important guiding principle for the therapeutic treatment.

The above mentioned conventional methods necessitate the pretreatment which requires at least three to four days in total, containing one to two days for the selective isolation of bacteria from a specimen, one day for proliferating cultivation, and one or more days for operation of fixation, and the culture thereof should be

continued in practice until the bacteria grow enough, therefore, the pretreatment may require one week or more days. In addition, any bacteria other than the causative bacteria may be contaminated during the culture step in some cases, and such contaminants may not be distinguished from the causative bacteria.

5 More importantly, as mentioned above, because many of the causative bacteria in the specimen to be proliferated and detected have been uptaked into phagocytes, and are already dead or in a bacteriostatic state due to the antibiotics administered, the number of the bacteria that can be grown may be small even under appropriate conditions for the culture of the causative bacteria, thereby, the actual
10 detection rate of bacteria is as low as about 10% when the clinical culture specimen is employed. In the other words, for the present, 90% of the examined blood from the patient clinically suspected as suffering from the infection of *Bacteroides fragilis* could not be identified for the presence of the bacteria after all, even though the culture is continued for further one or more days.

15 Although the determination of the causative bacteria and selection of the antibiotics suitable for killing the bacteria as quick as possible have been eminently desired, in light of the present situation as above, the presently employed practice depends upon a therapeutic treatment which is initiated when the infection of *Bacteroides fragilis* is clinically suspected without awaiting the results of the detection
20 of the causative bacteria. That is to say, a trial and error method has been practiced wherein an antibiotic having the effectiveness for the widest range spectra against many kinds of bacteria is administered first, and next, if the antibiotic is shown to be not effective in one or two days, another antibiotic will be tested. In such a therapeutic method, the infection of *Bacteroides fragilis* may rather be drawn as the
25 replacement of bacteria.

Moreover, when the method for detecting the bacteria in the specimen by staining them is carried out, skilled experiences are necessary to rapidly distinguish the bacteria based merely on the shapes seen under a microscope, because the components of the host tissue may also be stained. In such cases, it may be difficult to
30 lead a final determination.

As stated above, although the infectious diseases caused by *Bacteroides fragilis* are the diseases of which rapid and accurate diagnosis has been required, the

conventional diagnosis method could not have complied with such demands.

[Disclosure of the Invention]

The present invention was accomplished in view of the above-described problems in this art, and is directed to probes which have the specific reactivities toward DNA or RNA derived from causative bacteria of infectious diseases, specifically *Bacteroides fragilis*, and to elucidation of the nucleotide sequences of the portions of the gene essentially derived from *Bacteroides fragilis*, which should be comprised in the probe.

Accordingly, the bacterial DNA still included in the bacteria but in the process of breakdown through phagocytosis by phagocytes can be significantly detected based on its specificity using hybridization method. Therefore, rapid and accurate detection of the causative bacteria of infectious diseases can be achieved without culturing and proliferation of the bacteria. Moreover, identification of the causative bacteria can be accomplished through DNA amplification using PCR method without the hybridization process when a primer is designed with reference to the nucleotide sequence information of the probes of the present invention.

In addition, the probe used for the hybridization may be labeled with non-radioactive agent. If biotinylated probe is used for example, the detection can be carried out in a general examination laboratory not having a facility for radioisotope handling. Thus, operation for the detection can be practiced in a rapid and simple way.

In one embodiment there is provided a purified and isolated continuous nucleic acid which is useful for diagnosing infectious diseases and is consisting of at least one nucleotide sequence selected from the group consisting of SEQ ID No: 1, SEQ ID No: 2, SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5 and SEQ ID No: 6.

The invention further provides a probe consisting of the nucleic acid as defined above.

[Brief Description of Drawings]

Figure 1 (a) is a drawing which shows the positions of the originated bacterial strains of the DNAs on each of the filters of dot blot hybridization, and Figure 1 (b) shows the results obtained by color development after the hybridization process using
5 each probe.

[Best Mode for Carrying out the Invention]

In order to explain the present invention in more detail, non-limiting Examples with respect to the probes which are derived from *Bacteroides fragilis*,

causative bacteria of infectious diseases are shown below.

Example 1: DNA probe derived from *Bacteroides fragilis*

(1) Preparation of DNA probes derived from the bacteria *Bacteroides fragilis*

5 Clinical isolate of *Bacteroides fragilis* was cultured overnight in BHI (Brain Heart Infusion) medium under an anaerobic condition (5% CO_2), then the cultured cells were harvested, and genomic DNA was extracted therefrom in accordance with Saito-Miura modified method ("Preparation of transforming deoxyribonucleic acid by phenol treatment", *Biochem. Biophys. Acta* vol. 72, pp.619-629 (1963)) in which 10 cell lysis step was carried out by adding N-Acetylmuramidase SG to the lysis buffer.

The extracted DNA was completely digested with restriction enzyme HindIII, then random cloned into vector pGEM-3Z. Six probes specific to *Bacteroides fragilis*, that is to say, the probes comprising DNA fragments which showed specific reactivities toward DNA included in natural *Bacteroides fragilis*, were selected from 15 thus obtained clones.

Thereafter, the selected probes were named: probe BF-7, probe BF-17, probe BF-21, probe BF-28, probe BF-34, and probe BF-35.

(2) Studies of species specificity of the DNA probes derived from *Bacteroides fragilis*

20 Interactions between each probes and DNAs from several kinds of causative bacterial strains of infections were studied as follows.

First, the clinical isolates and deposited bacterial strains as listed in Table 1 below were prepared. In order to obtain the sources of Human genomic DNA in Table 1 and a control sample, leucocytes which were collected from four healthy adult 25 men, and *Escherichia coli* K-12, JM109 transformant containing plasmid pGEM-3Z were respectively prepared.

Table 1

Bacteria No.	Abbrev.	Name	Origin
1	BF	Bacteroides fragilis	Clinical Isolate
2	BT	Bacteroides theotaiotaomicron	Clinical Isolate
3	BV	Bacteroides vulgutus	Clinical Isolate
4	SA	Staphylococcus aureus	ATCC 25923
5	SE	Streptococcus epidermidis	ATCC 12228
6	EC	Escherichia coli	ATCC 25922
7	KP	Klebsiella pneumoniae	Clinical isolate
8	EBC	Enterobacter cloacae	Clinical Isolate
9	EF	Enterococcus faecalis	Clinical Isolate
10	PA	Pseudomonas aeruginosa	ATCC 27583
11	HI	Haemophills influenzae	Clinical Isolate
12	HPA	Haemophills parainfluenzae	Clinical Isolate
13	SP	Streptococcus pyogenes	Clinical Isolate
14	SAG	Streptococcus agalactiae	Clinical Isolate
15	SPN	Streptococcus pneumoniae	NYSDH DP-2
16	HUM	U937 Human Genomic DNA	

[ABBREVIATION]

NYSDH : New York State Department of Health (Albany,New York,USA)

Thereafter, the DNAs included in each of the clinical isolates were extracted according to the method described in Example 1(1), then the aliquot of the extracted DNA (e.g., 10-100 ng) was spotted onto a nylon filter. After denaturation with alkali, the filter was subjected to dot blot hybridization. The human genomic DNA sample was prepared from the leukocyte obtained as mentioned previously using Saito-Miura modified method (*supra*). A control sample was prepared from *Escherichia coli* K-12, JM109 transformant containing plasmid pGEM-3Z using the method for preparation of plasmid DNA described in the following Example 2(1). Hybridization was then carried out overnight using a Digoxigenin-11-dUTP (BRL) labeled DNA probe which was derived from the *Bacteroides fragilis* under a hybridization condition of 45% formamide, 5 x SSC, at 42℃ according to Manual by Maniatis (T. Maniatis,et al., "Molecular Cloning (A Laboratory Manual Second Edition)"., Cold Spring Harbour Laboratory (1989)).

After overnight hybridization was completed, the samples were washed two times with 0.1 x SSC, 0.1% SDS at 55℃ for 20 min. according to the manual, followed by color development and detection using Anti-Dig-ALP conjugates (BRL), thus results of hybridization were revealed. These results are shown in Fig.1, wherein Fig 1(a) illustrates the positions of the originated bacterial strains of the DNAs on each of the filters of dot blot hybridization, and Figure 1 (b) illustrates the results obtained by color development after the hybridization process using each of the above mentioned probes BF-7, BF-17, BF-21, BF-28, BF-34, and BF-35.

The experimental results with respect to the reactivities between each probes and DNAs from each of the clinical bacteria strains are shown in Table 2 below.

Table 2

Bacteria No.	Abbrev.	Name	Probe (Denotation: BF-) 7	17	21	28	34	35
1	BF	Bacteroides fragilis	+	+	+	+	+	+
2	BT	Bacteroides theotaiotaomicron	–	–	–	–	–	–
3	BV	Bacteroides vulgutus	–	–	–	–	–	–
4	SA	Staphylococcus aureus	–	–	–	–	–	–
5	SE	Streptococcus epidermidis	–	–	–	–	–	–
6	EC	Escherichia coli	–	–	–	–	–	–
7	KP	Klebsiella pneumoniae	–	–	–	–	–	–
8	EBC	Enterobacter cloacae	–	–	–	–	–	–
9	EF	Enterococcus faecalis	–	–	–	–	–	–
10	PA	Pseudomonas aeruginosa	–	–	–	–	–	–
11	HI	Haemophills influenzae	–	–	–	–	–	–
12	HPA	Haemophills parainfluenzae	–	–	–	–	–	–
13	SP	Streptococcus pyogenes	–	–	–	–	–	–
14	SAG	Streptococcus agalactiae	–	–	–	–	–	–
15	SPN	Streptococcus pneumoniae	–	–	–	–	–	–
16	HUM	U937 Human Genomic DNA	–	–	–	–	–	–

[REMARKS]

+: hybridization signal detected

-: hybridization signal not detecte

As is evident from the Tables 1 and 2 above, all of the present probes showed reactivities only to the DNA derived from *Bacteroides fragilis* , while no reactivity (i.e., hybrid formation) was observed toward the DNAs from the every other bacterial species in the genus *Bacteroides,* as well as the DNAs from the bacterial species other than genus *Bacteroides.* Thus, the specificity of the probes was demonstrated.

Example 2: Analysis of the Base Sequence

Each of the base sequences of the DNA probes (six probes in total) of which species specificity was demonstrated in Example 1 as above was determined according to the following procedure.

(1) Preparation of Plasmid DNA

Escherichia coli K-12, JM109 transformant, wherein the sub-cloned insert fragment (to be sequenced) is contained in pGEM-3Z (Promega), was inoculated into 5ml of Luria-Bactani Medium (bacto-tryptone, 10g/1L; bacto-yeast extract, 5g/1L; NaCl, 10g/1L; adjusted pH to 7.0 with 5N NaOH)and cultured overnight.

The culture liquid mixture was centrifuged (5,000rpm, 5min.) to collect the bacteria. One hundred μl of a solution of 50mM glucose/50mM Tris-HCl (pH8.0)/10mM EDTA containing 2.5mg/ml of lysozyme (Sigma) was added to the precipitate, and left at room temperature for 5 minutes. To the suspension, 0.2M NaOH solution containing 1% of sodium dodecyl sulfate (Sigma) was added and mixed. One hundred and fifty μl of 5M potassium acetate aqueous solution (pH 4.8) was further added thereto and mixed, then cooled on ice for 15 minutes.

The supernatant collected by centrifugation (15,000rpm, 15min.) of the mixture was treated with phenol/$CHCl_3$, and ethanol of two times by volume was added thereto, then the precipitate was again obtained by centrifugation (12,000rpm, 5min.). This precipitate was dissolved in 100μl of a solution of 10mM Tris-HCl (pH7.5)/0.1mM EDTA, followed by addition of 10mg/ml RNase A (Sigma) solution, then the mixture was left at room temperature for 15 minutes.

Three hundred μl of 0.1M sodium acetate aqueous solution (pH 4.8) was added to this mixture and treated with phenol/$CHCl_3$, then the precipitate was obtained therefrom by adding ethanol to the supernatant. This precipitate was dried

and dissolved in 10 μl of distilled water to give a DNA sample.

(2) Pretreatment for Sequencing

Pretreatment for sequencing was performed with AutoRead™ Sequencing Kit (Pharmacia).

5 Concentration of DNA to be employed as a template was adjusted to 5-10 μg in 32 μl of a solution. Thirty two μl of the template DNA solution was transferred to a mini-tube (1.5ml, Eppendolf), and added thereto 8 μl of 2M NaOH aqueous solution, then mixed gently. After instant centrifugation, it was left at room temperature for 10 minutes.

10 Seven μl of 3M sodium acetate (pH 4.8) and 4 μl of distilled water were added, followed by 120 μl of ethanol, and after mixing, the mixture was left for 15 minutes on ethanol/dry ice. DNA which was precipitated by centrifugation for 15 minutes was collected, and the supernatant was removed carefully. The precipitate thus obtained was washed with 70% ethanol and centrifuged for 10 minutes. Then,
15 after the supernatant was carefully removed again, the precipitate was dried under the reduced pressure.

The precipitate was dissolved in 10 μl of distilled water, then 2 μl of fluorescent primer (0.42 A_{260} unit/ml, 4-6 pmol [Fluorescent Primer; Universal Primer: 5'-Fluorescein-d[CGACGTTGTAAAACGACGGCCAGT (SEQ ID NO: 7)]-
20 3' (1.6pmol/μl, 0.42 A_{260} unit/ml); Reverse Primer: 5'-Fluorescein-d[CAGGAAACAGCTATGAC (SEQ ID NO: 8)]-3' (2.1 pmol/μl, 0.42 A_{260} unit/ml), and 2 μl of annealing buffer was added thereto, and mixed gently.

After instant centrifugation, the mixture was heat-treated at 65℃ for 5 minutes and rapidly transferred to a circumstance of 37℃ and kept the temperature
25 for 10 minutes. After keeping the temperature, it was left at room temperature for more than 10 minutes, and centrifuged instantly.

Then, the sample was prepared by adding thereto 1 μl of elongation buffer and 3 μl of dimethyl sulfoxide.

Four mini-tubes have been identified with one of the marks of "A", "C", "G"
30 and "T", and, according to the respective mark, 2.5 μl of A Mix (dissolved ddATP with dATP, dCTP, c^7dGTP and dTTP), C Mix (dissolved ddCTP with dATP, dCTP, c^7dGTP and dTTP), G Mix (dissolved ddGTP with dATP, dCTP, c^7dGTP and

dTTP), or T Mix (dissolved ddTTP with dATP, dCTP, c^7dGTP and dTTP) was poured into each identified tube. Each solution was preserved on ice until use, and was incubated at 37℃ for one minute or more before use.

Two μl of diluted T7 DNA polymerase (Pharmacia; 6-8 units/2 μl) was added to the DNA sample, and completely mixed by pipetting or mixing it gently.

Immediately after completion of the mixing, the mixed solution was distributed to 4.5 μl of the four types of the solutions respectively which had been incubated at the same temperature. Fresh tips were used for each distribution.

The solutions were kept for 5 minutes at 37℃, then 5 μl of termination solution was added to each reaction mixture.

Fresh tips were also used for this step. Immediately after incubating the solution for 2-3 minutes at 90℃, it was cooled on ice. Four to six μl of the solution per lane was applied for the electrophoresis.

(3) Sequencing on Base Sequences

Sequencing on the base sequences of the probes disclosed in Examples 1 and 2, having the specificity toward DNA from *Bacteroides fragilis* was performed using A.L.F. DNA Sequencer System (Pharmacia) under a condition of the electrophoresis process of 45℃ for 6 hours. Primers were serially designed based on the sequences elucidated from each of the upstream and downstream sequences, and the above described procedures were repeated.

Consequently, all of the entire base sequences of the probe BF-7 (SEQ ID NO: 1), probe BF-17 (SEQ ID NO: 2), probe BF-21 (SEQ ID NO: 3), probeBF-28 (SEQ ID NO: 4), probe BF-34 (SEQ ID NO: 5) and probe BF-35 (SEQ ID NO: 6) were elucidated.

[Industrial Applicability]

Using the probes according to the present invention, the causative bacteria which were incorporated into the phagocytes can be rapidly and accurately identified directly without proliferation of the bacteria by for example, a hybridization method. In other words, the diagnosis wherein the probes of the present invention are used enables the identification of the causative bacteria with single specimen, further, the necessary time for diagnosis can be diminished to approximately 1 to 2 days, while

the conventional method with low detection rate requires 3-4 days, and the resulting detection rate is remarkably improved. Therefore, the present invention provides guiding principles of the therapeutic treatment for the infectious diseases caused by *Bacteroides fragilis* , in addition, the effective treatment in an early stage of the
5 infection can be adopted to the patients, which may lead to a reduction of the mortality.

Additionally, in accordance with the present invention wherein the base sequences of the probes which specifically react with the DNA derived from *Bacteroides fragilis* among other several causative bacteria of the infectious diseases
10 were elucidated, artificial preparation of these probes has become feasible. Moreover, a part of the information of the base sequences provided herein may be utilized to produce primers, which are useful for rapid diagnosis through amplification of DNA of causative bacteria contained in the clinical specimen by a PCR method.

Furthermore, the rapid identification of the causative bacteria may be carried
15 out by comparing the base sequences of the genomic DNA from the clinical specimen with the base sequences provided by the present invention.

As stated above, the present invention provides the desired probe for the diagnosis of the infections, besides, outstanding utilities as guiding principles for the manufacture of the primers for PCR as well as standard sequences which are suitable
20 for the comparison of genomic DNA contained in the clinical specimen can be expected. Moreover, the present invention may exert beneficial effects by providing valuable clues for preparation and development of the novel probes which specifically react with the DNA from the causative bacteria of the infectious diseases.

Further, the base sequence disclosed in the present application was obtained
25 by random-cloning of the genomic DNA from the clinical isolates, therefore, the utilities of the base sequences of the present invention should be encompassed to the complementary strand thereof.

Additionally, it may be presumed that DNA obtained from the wild strains might contain the mutated portion. However, as apparent from the disclosure of the
30 Examples above, such mutated DNA portion would not affect the utilities which should be derived from the present invention, comprising the specificity of the probe of the present invention in the hybridization procedure for the diagnosis of the

infections, and usages of the information on the base sequences disclosed in the present application for designing the primer to be employed for the PCR techniques with the aim of a rapid diagnosis of the infections.

SEQUENCE LISTING

GENERAL INFORMATION

APPLICANT: Fuso Pharmaceutical Industries, Ltd.

TITLE: Probes for the Diagnosis of Infections Caused by
Bacteroides fragilis

NUMBER OF SEQUENCES: 8

CORRESPONDENCE ADDRESS: Kirby Eades Gale Baker
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CANADA

COMPUTER-READABLE FORM
COMPUTER: 1.44MB
OPERATING SYSTEM: IBM AT compatible MS/DOS
SOFTWARE: ASCII

CURRENT APPLICATION DATA
APPLICATION NUMBER:
FILING DATE: September 23, 1999
CLASSIFICATION:

PRIOR APPLICATION DATA
APPLICATION NUMBER: JP 9/71079
FILING DATE: March 25, 1997
CLASSIFICATION:

PATENT AGENT INFORMATION
NAME: Andrew Bauer-Moore
REFERENCE NUMBER: 43764-NP

INFORMATION FOR SEQ ID NO: 1
SEQUENCE CHARACTERISTICS
LENGTH: 2184 base pairs
TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULAR TYPE: Genomic DNA
ORIGINAL SOURCE:
ORGANISM: Bacteroides fragilis
STRAIN: Clinical Isolate BF-7
SEQUENCE DESCRIPTION: SEQ ID NO: 1

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AAGCTTCTAC ACGCTTCAAT TCTTGAAACA CAAGTTCTAC CGCAAGCTGC GGATTTACTT     60
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TGGGCGTGGT GTCCTTTCTC TTTCGGGGGC TGTACTGCCC CTGCTTATAG GTGTAATCCT    120
CCGTCTTTGG GTTTGAGGGT GTTTCCATAA TTCCTGCTGA TTGAGTTATA CTTCAATTTT    180
TTCTGTGTCT TCTCGATATG GGCAAGCGTA TAGCCTTTCC CTATCTCGCT TGCCTTGTAT    240
TCCGTTCCGC TCCGGGCTTT CACGTAGTAG CCGTTGAGTT TGCCTGTACT TGCCCGGGCT    300
TCCCTTACCT TGAAGCCCAG TTTTCCGAGT TCCCGGCTGA ATCCGGCAAG GTCAAACCCC    360
TGCATCCGTA TAAGAACATC ATCCATAGCC TGTTTTATCT CTTCCCTGTT GGCTTTGCCT    420
ATATCCTTTG ACTGTACCAA GTTCCGTTCT CTGGCTATAC TGTTGGCAGC TTCCGTAGCT    480
CTCTTCCCTA TCCAGTTATC CTGGTATAGT TCTCCCGATA ACGACACACG GTTTGCCAGT    540
ATATGCAGGT GGGCTTGCCT GCGGTTCTTC TCCGTTCCGC TGTGCTTCAC AATGATGTAC    600
TGGTGGTTCA TCAAACCCAT GCGCTGCATG AAGTCATTAC CGAGCTTCGC CCAGTCCGCA    660
TCGGTCATGC CTGCACTTTC TTCCACTGAA GGGCTGACCT CAAACCGAAG CAGTTGTTCT    720
TTACGTTCGG AAAATCAACA AAGTAAGGTT TCATCTCCTG CACCATATCT TCGCCTGTGC    780
AGCCGAAAAG CTCATGGCGG TGTATCTCGG TGGCTGCTGC CTCTCCGTTT ATCTCTTTGG    840
CAAGGTCGTA CTCCAGTGCT GCCGTGCCAT GAGATATGCT CTTTCCTTTT GCTATCATCG    900
TGAAAGTATC TTTTTAAGTT CATTGATTAG TGCCTGGTTC TCCTCGAACA CTTCCCTGTA    960
CTGGCGCCCA CCGAAGTAGT TGTTCAGACG CTGGAGTGTT CCTTTCAGTC GGGCTATCTC   1020
CCGAAACAGT TCCCTTTCTT CTTCCGTGTA CCTCTCCTTG GGTCGTCCGC CCAGTGCGAG   1080
TGTACGGCAG TACTCCGATA TGCTGATGCC GCATCGGGCT GCCTGTTCCG CAAGTGCCGC   1140
CTTTTCAAGT GCGGTGCAGC GGAATGTTAT CTTCTCCGTT CGATTTACTT TCATGTCGCA   1200
AAGGTAATCT TAAAATTCTT TTGAGCAAAG CGAGAAAGGC AAGAATGTCC GACAAGGACA   1260
CTTCTTGCTA TATATACACA TTCCGCATGG GAATACACAC AGCGCTAACA CACTGCATAG   1320
CCACACACTT TTTTTTCTTT TTCCCTGCCA TTTCCACAAC GGAACACGGA GAGCGGTCAA   1380
GGGTTCGAGC GGAAAAAACC GCACAGCTTC ACTGTGCGGT TTTTTCCGCT CCGAACTTGC   1440
GAAGCCCTTG ACGGCTTCGG AGTGTGGAGT TACCTTTGCA GACCGAAAAG AGAAAAAACA   1500
CAGTTTCGCT TCACTAACGC TGACTGGTCA AAGGATACCC TGTTCAGATA TGATTTTTTT   1560
AAGAATAGAT ACACTTACGG TAGAGAAGTC TGACTTATCA TAGACATCCA CAAGATAAAC   1620
ACGACCTTCA CTCTCCGATG CACAAATCGT GTATGTGATA ACACGTGCTC CCCCAGACTT   1680
ACCCTTTCCC TTAGAAACAA TGGCAAGTCT TATTTTACGG ATACCAGGGC TTAGTTCATC   1740
CCCCTGCATG GGGTTCTTTT CCAATGACTC GATAAAATCC TTCATGTCAG CCTTAAAAGA   1800
CTTATACCTT TTTGCAAGGA TTTTCGCTTC CCTCTCAAAA TGTGGGGTGG TCTTTACTTC   1860
AAAGCTCATC AAGAAATGCG TTTGCGCTCT TGGCTGGCTG CTCACCAGCT TCGATTCGTT   1920
TCACTTCATT CAAGCCCTCT CTTATTCGAG TGGCTATTTT TTCTTCTACG GACACTGGCA   1980
CCAATTTAAA ATCACCGATA CGTGAAGTCA GAAAGACGGA TTCTCCCTGA ATAGCACGAA   2040
GAAGGGTCGC TGTCTGATTC GTCCGAAATT TGCTCAATGG AATTACTTGC ATATATCTTA   2100
AACTTTTTGA TTGTGATACA AATGTAACCA TTGTCACCGA TATTGTAACC AAATTAATAT   2160
ACTTTAACAC GCAAAAAAAA GCTT                                          2184
```

INFORMATION FOR SEQ ID NO: 2
SEQUENCE CHARACTERISTICS
LENGTH: 2163 base pairs
TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULAR TYPE: Genomic DNA
ORIGINAL SOURCE:
ORGANISM: Bacteroides fragilis
STRAIN: Clinical Isolate BF-17
SEQUENCE DESCRIPTION: SEQ ID NO: 2

```
AAGCTTACGG CAGCGGGCGG CGCTACTCCT TTCTTCTGCA CGACTGCTTC CGGATCCGGA    60
TCGGCCGGCA CGATGTCCCT GACGGATGGC GCTTTGTCCG GACTGGCCGC GGTGGGTGAA   120
ATCTCTTTTA CCCCATCGGC CCGTCCGCAA CAGGTCGGCA CCTCGGGCCC CGTTACAGTG   180
GCCTACGGGC TGGTGGCTCC GAAGCTGAGG CGGCTTCGGG CATGCGGGTG ACGTTGACGC   240
TGACACTGGG TGAACAAAGC TCGGACGCTA CCGAACGGGA TCTTACCCTC TCGACCGATG   300
CCTTCAACCC GGCATGGCAG AAAGGATACC GCTACGTCTA TACCTTGACG CTCGGCGAAC   360
GGGGAATCAG CCTGCAACCT GTCGATATAA AAGGGTGGAC GGAAGTTTCC GAGGAGAGCA   420
GTGACGTAGA TCCCGGCTGG CAATGAAACA AATGATTCAG AATGACAAAT GATTCAGAAC   480
AATAAATGGT TCAGAATGAC AAATGGTTCA GAATGACAAA TGACAAATGA TTCAGAATGA   540
ATGATTCAGT ATGAAAACAG ATAGATTATA CCAAACGGTT TTAGGCTGCC TGCTGCTTAC   600
CGCAGTTGTT CGGACAATGC GCTGACCCCT TCGTTGCCCG AAGACCTCCG CTTACGGTGT   660
CCGCCGCGAT CTGCGCTTCC GGTGAAGGCC GGGAGTCTGA GACCCGGGCG GCCGTGGCAG   720
CCGGGACCCG TGCCGTGGCA GCCGACAACG GTTACGACCG CAGCACCTTT GCCGCAGTGA   780
CAAAATCCGT ATCATCCGCT CGCGGAACGG CTCGTCATCC ACTCCGGTGG ACTATATATT   840
GAACTCCGCT TCTTCCGGCA ACAGTACGGG CGAGTGGAAA CCTTCGGTGA CGGGTACGGA   900
GCTGCTCGTT GAATCCGGTG CCACCTACCA GGCGAGTTAC CCTATCGAAT ACTCGGGTAT   960
CCGTGCCGAC CAGCGAAAGG CGGGAGGTGA GGACTACCGC CTGAGCAATC TGCTGGAGAC  1020
TCCTGAGAAG GTGGCGATCG GCCGTGACGG AACCCTCTCC TTTACAGGCG AAAGCCCTTT  1080
GTGCACAAAG GCGTGAAGCT GACCCTGAAA TTTTCGAGAA AGCATACACT GTCCAAGGAT  1140
TTTACGAGTA TGACCGTAAC CGGGAATGGG CTTTACTCCG GAGAAGCAAG CAAGGATGAA  1200
ACCGTCTACC TGTATCACCC CGGCGGTACA GACAAAGAGC AATATACCTG GCATGGCATT  1260
ATCGCCCCGC TTACCTTCCC AACAGTCGAT ACAGGTATCG GTGACGGATG CTAACGGAGT  1320
GGTCTATGAC GTCACCCTTA TTTGCGCAAG GGCGGCGAAC AGTCACTACA CCTATACGCT  1380
CACCCTGAAA AATGATGTGC TGGTTCCCAC CGGTCAGGAG ATCAAAGAGT GGCAAAGCGG  1440
GGATTCGCAT ACAGGAACCT TAAGTTGATG TAACTTAGTT TGATGTAATA TTAGTTGATG  1500
TAATATTAGT TTGATGTAAC CCCATGAAAA AGAAAAAGAA TAGAATCCTA TCGGTATCCG  1560
GCGCGGCTCT CTGCCTGTTC TTCCCGGTTC TGCTGGCCGC CTGCTCGCAA GAGGATGCTT  1620
TGCCCCGTCC GTTGGAAGTA TCGGCGGAGG TGGGGAATCC CGCTACCCGT GCGGCGGCAG  1680
ACTCGCATGC CGATGATTAT GATAAAAGTG AATTTGTAGC GGGTGATGTC ATCCGGATTA  1740
CGGACGGCAC GAAAACTGCC GACTACCAAC GGGTGGTCAC CGGCACAACC GGCACCTGGC  1800
```

AACCCGCAAG CGGACAAACA GCGCTCACCA CCACCGGTAG TGAAACGTTT ACGGCTTCGT 1860
ATCTACGGCG TTCACCCGGA TTCTAACCGA TCAGCGCACA GCCACCAATT TCTGGCAAAG 1920
CAACCAGCTG ACGGCTAAAG GAGTACTCGA TGGCAATAAG GCTACGTTCT CGTTCGCTCC 1980
GGAAGCGGCG AAGGTGACCT TGGTGGTGAA GTATGGAAAT AGTGATAGTG ACAAGAAGCT 2040
TCATTATGGC CAACAACGTT CCTGTTGTTT TAGACAGAGA AGCCAAATCA TAAATATCCG 2100
TCGGCTTCAC TTTCTCGCTA TTTGCATCTG TATGGTATCC GAAGCACCGG TCATACAAAG 2160
CTT 2163

INFORMATION FOR SEQ ID NO: 3

SEQUENCE CHARACTERISTICS

LENGTH: 1657 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULAR TYPE: Genomic DNA

ORIGINAL SOURCE:

ORGANISM: Bacteroides fragilis

STRAIN: Clinical Isolate BF-21

SEQUENCE DESCRIPTION: SEQ ID NO: 3

AAGCTTATCT TATGGACCGT ACCCGAAGGA GAATTGCTTG AAGAAGCGAT TGAATGGTGC 60
CGCCAACGGG GAGTCTTTTT CTATTCTGTC AACAAGGACT ATCCGGAAGA AGAAAAGAGT 120
CATAACGGAT TCTCCCGTAA ACTGAAAGCA GACCTGTTTA TTGATGACCG GAACCTGGGA 180
GGTTTGCCTG ACTGGGGAAC CATCTACCAG ATGATCCATG AACAAAAGCC ATACGAACCT 240
GTTCTATGTG ACAGGCAGAA ACCGACCGGC GATTTAAGCT GGATAGAGAA ACTGCTCGGC 300
AAACGTAACA AATAAAGAAA GAGGTTGACA ATGAACAATC ATGTAGTAAT TATGGCCGGT 360
GGCATAGGAA GTCGATTTTG GCCCATGAGT ACACCGGAAT GTCCCAAACA ATTCATAGAT 420
ATATTGGGAT GTGGAAAAAC ACTGATTCAG CTAACTGTAG AGAGATTCGG TAATGTTTGT 480
CCACAGGAGA ACATGTGGGT GGTCACTTCG GAAAAGTATA GAGATACTAT TCGGGAGCAA 540
CTGCCGGGTA TCCCGGAAAG TAATATACTG GCAGAACCCT GTCCCAGAAA TACAGCTCCC 600
TGCATTGCGT ATGCCTGCTG GAAAATAAAA AAGAAATATC CGGAAGCCAA CATTGTCGTG 660
ACTCCTTCCG ATCAAGTGGT AATCGATACC ACTGAATTTC GCAGGGTGAT TGAGAAAGCG 720
CTTTTGTTCA CTGATAAAAG CAGTGCTATC ATCACATTGG GAATAAAACC CGCCCGTCCG 780
GAAACCGGAT ATGGATATAT CGCCGCAGGT GAACCGATAA CGAGAGACAA AGAAATATTC 840
CACGTAGAAG CATTCAAGGA AAAGCCTGAT AAAGAAACTG CTGAAAAATA TCTGGCAGCA 900
GGCAACTACT TCTGGAATGC AGGAATATTC GTTTGGAATG TGAGAACGAT CACAGCCGTA 960
ATGCGGGTAT ATGCACCGGG GATAGCTCAG ATTTTCGACC GGATATATCC CGACTTTTAT 1020
ACAGAACGCG AGGAAGAAAG TGTGAAGAAG CTATTCCCCA CTGCCGAAAG TATCTCGATA 1080
GATTATGCAG TGATGGAAAA AGCGGAAGAG ATTTATGTAT TACCTGCCCA AATGGGGTGG 1140
TCGGACTTAG GTACCTGGGG AGCATTACAC ACCTTGTTGC CAAAAGATAA AGAAGGAAAT 1200
GCAACAGTAG GACCGGATAT CCGGATGTAT GAAAGTCGAA ACTGCATGGT GCATGCCTCA 1260
CAGGAAAAAC GAGTAGTCAT ACAAGGGCTG AACGATTACA TCATAGCCGA AAAAGACAAT 1320

ATATTATTAA TATGCCAGTT ATCAGAAGAG CAACGAATTA AAGATTTCTC AAAAGAATAA 1380
ATGTTGATCC CTTTCAATAT TTATAAATCC CGGTTATAAT ACCGGGTGTA GGGGCGTCCC 1440
GATGCGAATC GTGACTGCTT TTATTTGTTT TGTTTGTGTG TGTTCCTCCG GCCAGGCGTG 1500
GTCGGAGGAA CATTTTTTTA TGCCCGTATT CTACAACTTG TGATTCAAGA TCCAATCCGA 1560
AAATTTCTTT AAAGACTATC TCGTCCAAGT CACCAATCTC AACCTTAAAA TGAAAGATTC 1620
ATACCAAATG TTTCAACGAT AAAGTATAAA AAAGCTT 1657

INFORMATION FOR SEQ ID NO: 4
SEQUENCE CHARACTERISTICS
LENGTH: 2079 base pairs
TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULAR TYPE: Genomic DNA
ORIGINAL SOURCE:
ORGANISM: Bacteroides fragilis
STRAIN: Clinical Isolate BF-28
SEQUENCE DESCRIPTION: SEQ ID NO: 4

AAGCTTTCCT TTCCTATCGT ATTTAAAAAC TCCTGCATCA CTACCGGCCA ATACGAAAAT 60
ATAGTCTTTA GTCACTGCCA TACTGAGTAT ACTGGCACGA ACCGGCAATT TCACAAAATT 120
ATCCAACTGG ACATACGAGA TGCTATCTAC CAATTCCTTC ACTGAGATGG AGTCCTCTCT 180
ATCTAAAACA CTGGTCATCT CAACAATCGG ACAATCAGAT GGCACCTGAG CTAACAAATC 240
AATCTTCTCT GAACGGGAAG AAGAGCAACC GATCAGATGC GTCAATATAC CACAATATAA 300
AACTAATCTA ATCTTGTTCA TTTTGATTTT ATATTTAAAT AAAAGTCCAA CATCTTATTT 360
CTAAATAAGT CAAAACCATA TAATTGTATA TACCGATTAA CCAAATAATC TTTAATACTT 420
TTTTTTTCTA ACAAATTCAT ACTCAAAGCA GTTATCATCT GCTGTGCTAA TATCTCAACA 480
TTAATAGAAC AAATCTCTTC CTGAGATATA TTAACTCTAA AAGGATAAGA TGGTCCAACC 540
ATTTCATTTA CTCCTGCTAC AGAAGTTGCT ACCAAAGGAA TTCCTCTCAT CATCATTTCA 600
ATCATTACAT AACTACATTG TTCTGTATAT GAAGCAATAA CCCCTAAGTC AGCCATAGAA 660
TAAATACAAT CCAATTTATC TTTCTCAACT CTACCAACAA ACGAAACTTT ATGCCATATT 720
CTTTCACACA GTTTCAAGTA AGTAGCGAAT TCACCATCTC CAACAATGAC TAAACGTACA 780
TTCTGTATTT TATCCTGTAC CATGCGAAAG GCTCGCAATA GTTCTTCTAT CCCTTTATTA 840
TGATCCAAGC GTCCTACAAA TAATATCAAA ATTTCTTTTT TACCTAGAAC CGGCTCCTTT 900
TCACATTCAT TATCTTGAAC AGATAGCTTA TGTGGCAAGC CATTTGCTAT ATATTTGAGT 960
TTAGCTATAG GGACTTGATA ATCATTTATC AGAATCTCTT TAGTACTTTT TGAAAGACAA 1020
ATAACATAGT CAACATAATT AAACATTTCT TTCTCAACCG TAAATGCATC AAACACATCT 1080
ATTTCCTTCA AACTCCTTTT GGTCGCATCT TGAGATAAAA TAAAATGGAA ATAAGTCCTA 1140
TTTCCCTTTA AAACAAAACA CCAATTAAGA TAATGCACGG TAAAACAAAC CACACATCTT 1200
ATCTGCCTGC TCCTCAATGA TCTTACAATT GCCACATCTT GGTAAAAATT CAAATGAAAT 1260
ACATTAATAT TAAATAAATC AATATACTCC TGAAGGATAT ATGATATATT TCGACAGTAC 1320
CTTTCATACA AAAAAGAATT ATCTTGAACA TCAACACTAG GAATAAGAAA TAGCCTTATA 1380

CCATTATTGG TTCTCATTTC AATATTTTCA ACATTTGCAC AAATCTGGAC TATTGTTAAA 1440
TTTAAGTCCG CACGTTCTCT TACCACTTTT GTCAACTGTT CAACATATGT TCCTACACCA 1500
TATTGTGAAG CTACACTTGA ATTATTAACG ATATAAATAT TCCTCATATC GAAGATTGAT 1560
CCCAAATTAT TTTTAATCCT TTTTTCCAAG ACAAATTATT ATCGCCATCA CTCCCTCTTA 1620
TGATCTCTGC CTGTATCTGT TGTATAGAAT AATTCACAGG ACAAGCTACA CATAATGCTT 1680
GAAGAAAAGC CGTATCAAAA AACGACTCTC TACCTATATC CAGTACTAAA CGAATACGTA 1740
TATATGAAGC TATACCAAGC AAGCCTGTTT CCAGAGAATG ATTATTCATT TTTTAAAAGA 1800
TTGTATTCCA ACAGCTTATT ATCAAGAGAT TTTAAAACGT CATCCACATT TCCATCTACA 1860
AATTTTTGCT GAAACAGATA TACTATTCCC CACCCAATGC CACATAAGCC ATCCGCAAAG 1920
TAAATCGGTA TATCTTCATG AACATCTTCA AAAACCTCAT CCAATAATTC ACCAGCAAAT 1980
TCCTCATATA AAGGGTTTTG TATATAACGG CTATAATGAA AGAAAAACAA GACAAGTCCC 2040
ATCTTGCCAT TAAAAAGTCC CAAACTTGGA AGGAAGCTT 2079

INFORMATION FOR SEQ ID NO: 5

SEQUENCE CHARACTERISTICS

LENGTH: 1847 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULAR TYPEGenomic DNA

ORIGINAL SOURCE:

ORGANISM: Bacteroides fragilis

STRAIN: Clinical Isolate BF-34

SEQUENCE DESCRIPTION: SEQ ID NO:5

AAGCTTATAA AGAAGATTAT ATCCAACGAT TATTCGTCTT TAAAATTATG GATTGGAGCT 60
GATGAATTCA AGGAATTGCT TTATCTGAAT CAGGAAGGCG GTTCAGAGGG CACTACAGAA 120
GTCTATGATT ATGATGGTAA CTATCAAGGA AATATTGCTG AATTGTATAA TATGCCATAT 180
CATTCTCAAT ATGTACATCG ACTGAATGAA AAATTCCTGG CTGCTTTTTC ACCATGGTGT 240
ATGCCATTGA CTGATAAGAA TTATTTTGGC GGAGCCGTAT TTTCAGAAAA GGGGGAAATG 300
GTACATCAGT TGAACTATTT TGTTCCGTCT GACACTTTGG CTTTATGTAA GGTTAGCTAT 360
TATAGTTTCA CTTATCAATC AGATGGAAGC ATGTTGGTTT GGAATAATGG GGGAAGTGGC 420
AGCCCAATTG TTCAGCCTTA TACCACTTTG TATAAGGTAA CTGTTGATTC TATTTTTCCG 480
GTGTATCATT TGTTTAATGG TAATACACGA GATAAAATAG ATCATGTTCA AGGAATTGGA 540
GTTAGTGGGA ACTCTTTGGC CATAGAAACA GATTCCAGTC TCTATTTATG TTGTTATCAT 600
CCAAAGGCTC CGCATCCGTG TCCAACTTTA TGCTTTATGC GATATGATAT AAAAAATAAG 660
GTTCTTCAGG GAGTCAATTA TCCTCCTAAT GGTATTTGGG GAGGATATGT AAACCATTTG 720
AATGGTGATA TTCCAATCCG TTTCCAGTAT TCGTTTCCTC TTCAAAAGGT TTATGTATCG 780
AGTATTAGTT CTGGCGAAAT AGAGCAATTG AGAAAGTCGG GGTATATCAA TGCAAATAGT 840
GATGAAACAT TAAGGAGTAA TAAATCTGGG GATAATCCCA TTTTGATATA TTATCATTAT 900
TAATTATTTA ACAAGGAGGT AAATATGAAA AAGCTAAAAG TTTTAAATCT CTCAAAAGGT 960
GAGCAGTTTA AGATTACGGG AGGGGCTGGT AGTTGTACCG GGCCTGGTAC TTGTCGCGAA 1020

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AGTAACTGTA CATGTACAGG TTGGTTAGTA AGTAACCATG CATGGACAGA AACAGATGCA   1080
AGTAAAGAAA CGCAGATGTC TTCTAACAAG GACGTTTATT CTTGGCAAGG TAACCCTGGA   1140
GCTTAGTTTA ATGTAGGTGG TAGTTTACTA CCACCTCTTT TATGTAAATT ATGAAATGAA   1200
ATCTATTATA TTTAAGGATC AATATAATTA TTGCTATTTG TATAGCTTTG GTAAGAAAAA   1260
ACTTTTACAG ATAACTCCTG AGGTTTATAA TATGCTTGAT GTTTATTGGG CTGAAGGGGA   1320
ATGTTGGGAA GGGGATAGTA ATACGGCGCG AACATGCTCT TTTTTTGAGA AATATGGTTT   1380
TTTGGATTTT GACAATATAA AAGTTGGTGC GGGTATAGAT GTAGAGGATA TAGAATATTC   1440
CATTGCAAGT GTACCAGTTA TAACATTTGA GTTGACTCAG AAATGTAATC TTGGTTGCAA   1500
ATATTGTGTG TATGGAGATC TATATGAGAC CGAAAAAGAT TGTGTTGGGA ATGAATTATC   1560
TTTTGATGTA GCAAAATCTG TCATTGATTT TTGCTTGAAT AAGGGGATGA GAAACCAATT   1620
AAGTGGTTTA AAAAGAACTA TTACAATAGG CTTTTATGGT GGAGAGCCTT TGTTGCGATT   1680
TGATTTGGTA CAGCAAATAA TATCTTATAC AAAAAGTCGG GAATCTGAGT TTCTTACATT   1740
TACTTATAAT ATGACAACTA ATGCCGTATT ACTAGATAAA TATATGGATT ATATTGTTGA   1800
GCAGGATATT TCTCTTCTTA TTAGTCTTGA TGGTGATAAG AAAGCTT                 1847
```

INFORMATION FOR SEQ ID NO: 6

SEQUENCE CHARACTERISTICS

LENGTH: 1673 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULAR TYPE: Genomic DNA

ORIGINAL SOURCE:

ORGANISM: Bacteroides fragilis

STRAIN: Clinical Isolate BF-35

SEQUENCE DESCRIPTION: SEQ ID NO: 6

```
AAGCTTTTCA TTATACCCCC CCAAGAAGGA AAGAAGGTCT TCATACCACC CAGATCATCT     60
ATAATCTGTT TCACCGCAAA TGTCTCACCC GTACGTTTAT TGTAAATGGC TGTTAATAGA    120
GGAGGCTTAG GCAGTTTACG CAGCTCCCAG TATGCCATAT ATTCATAGAC AGATAAAAAA    180
ACAAAACGCT CTGTTTCAAA AACACCGGAT ACTATATATT TGTGATCCAG CATTTTAATC    240
ATTTTTGCAT TTTCCAAGTT GCCCGATTCC CAACATTTAA AAGCATCAGA AAATAATCCA    300
TCTTCATATA GATACTGGGT AGGAAACCGT AATTCTTCAT CCAACTCCAC CACCCAGCGA    360
GGTACCAGGG AAGTGGGGGT AACAGTAAAA ACTGTATCCG TATAAAAATT ATAAAATAAG    420
GTCGTATCTT TATAGTAGCT CGTATACTTC ACACTCCCCA TGGACGGAGC GATCCCTTTC    480
CTGTTCCACG GCATCATACT GCGTTTGGCT ACAAACTCGT AATCCGTATT TATGAAATAA    540
ACCGTATCCG GAGAATTATA AAACATCAGG TTTTCTCCTG CCGGAGCAAA GCAGACCTGA    600
TTGACCAAAT GTCCAACCGG TGCAGTGTCC ATTGTTTTCT GGGTAAACCA GCTTAGCTCT    660
TTCCCCGTAC ACTTCCCCCG GTCATCATAT TCTATTATTT TAGAGGCATT CCCATAGAAA    720
TGATTATTCA AATATACTAT TTCGGCCACA TGCGCATAGA TATCTTCACA AGCCCCTTGA    780
CCCTGGCTCC CAATACGGAA CAAGAACTGC CCATTCCAAT CAAAACAATA GTTTAAGTAA    840
AATATGTATT GGGGAGAAAA GGTCAAACGC TGATAATTAC CTAACATACA ATTCGGATTC    900
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GTTTCCAAAG GAATATAACT GACACTATCC ACCAGCTCCG AAAGCATCAT TTCCTCCCGA    960
ACATTCTTTA TCGCAGTTGA CAAATGAATA ATATTATCCC GAACAACAGG ATTAGGATCC   1020
ACTGTTTTTT TGTTACTACA AGCCGTGAAG CCCAATACCA TAACACCCAG TATCAATATC   1080
ATTCGCATAA AAAATTCCAT ATCCACCTAT TCTTTAAATT AGCAATTACA AGAACCGGAT   1140
TTTGCTCCTC ATCCAGTGAA TCGGTAAGCA TAACCAAACG CTCACTCACA ATTCCTCCCT   1200
TTTCTTTCTT CTCTTGAATA TATTCCTTCA ACTCAAACGG CCAAACCGAA GAAAAAAAAC   1260
TTTAGATTCA AAATAATAAT TCTTCTATTT TAAAGTTGCA ATCATCAAAA TCGGATTGCT   1320
ATCCTCAGTC GCACTTTCCA AAACTCGGAT TAGCTCCTTT TTCTTGGACT CATCTATTAC   1380
GGTAGATGAT TTTATTTGGT CGATATCAAT CCAGTTCTCA TCACCGCCGG GTTCTAATAT   1440
ATCTACCCAG TATTTACCGG AAGAACGGCT GTCAACAGTA AAATCACCTC CACCTAAATC   1500
ATTATCTAAC ACAAAATCAC GTTTCATTTG GCGCAAAGGA AAGCTAAACC AGGGGACATC   1560
ACGCTCGGTT ATTGCACTTT GTCGCTTCTG CAGTCTAACA TTTCCATCTT TTTTATTATA   1620
GCAATAGGTA TATACCTCCT CGCCTTTACT ACCCACCAAA TAAATGAAAG CTT          1673
```

INFORMATION FOR SEQ ID NO: 7

SEQUENCE CHARACTERISTICS

LENGTH: 24 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: Other Nucleic Acid (Synthetic DNA)

SEQUENCE DESCRIPTION: SEQ ID NO: 7

```
CGACGTTGTA AAACGACGGC CAGT                                          24
```

INFORMATION FOR SEQ ID NO: 8

SEQUENCE CHARACTERISTICS

LENGTH: 17 base pairs

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: Other Nucleic Acid (Synthetic DNA)

SEQUENCE DESCRIPTION: SEQ ID NO: 8

```
CAGGAAACAG CTATGAC                                                  17
```

Claims:

1. A purified and isolated continuous nucleic acid which is useful for diagnosing infectious diseases and is consisting of at least one nucleotide sequence selected from the group consisting of SEQ ID No: 1, SEQ ID No: 2, SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5 and SEQ ID No: 6.

2. The nucleic acid according to Claim 1 which is in the form of polynucleotide.

3. A probe consisting of the nucleic acid according to Claim 1 or 2.

4. The probe according to Claim 3 which further comprises a detectable label.

5. The probe according to Claim 3 or 4 which hybridizes to genomic DNA of *Bacteroides fragilis*, but fails to hybridize to genomic DNA of *Bacteroides theotaiotaomicron* and *Bacteroides vulgutus*, under the condition of:

hybridizing overnight at 42°C in an aqueous solution comprising 45% formamide and 5 X SSC; and

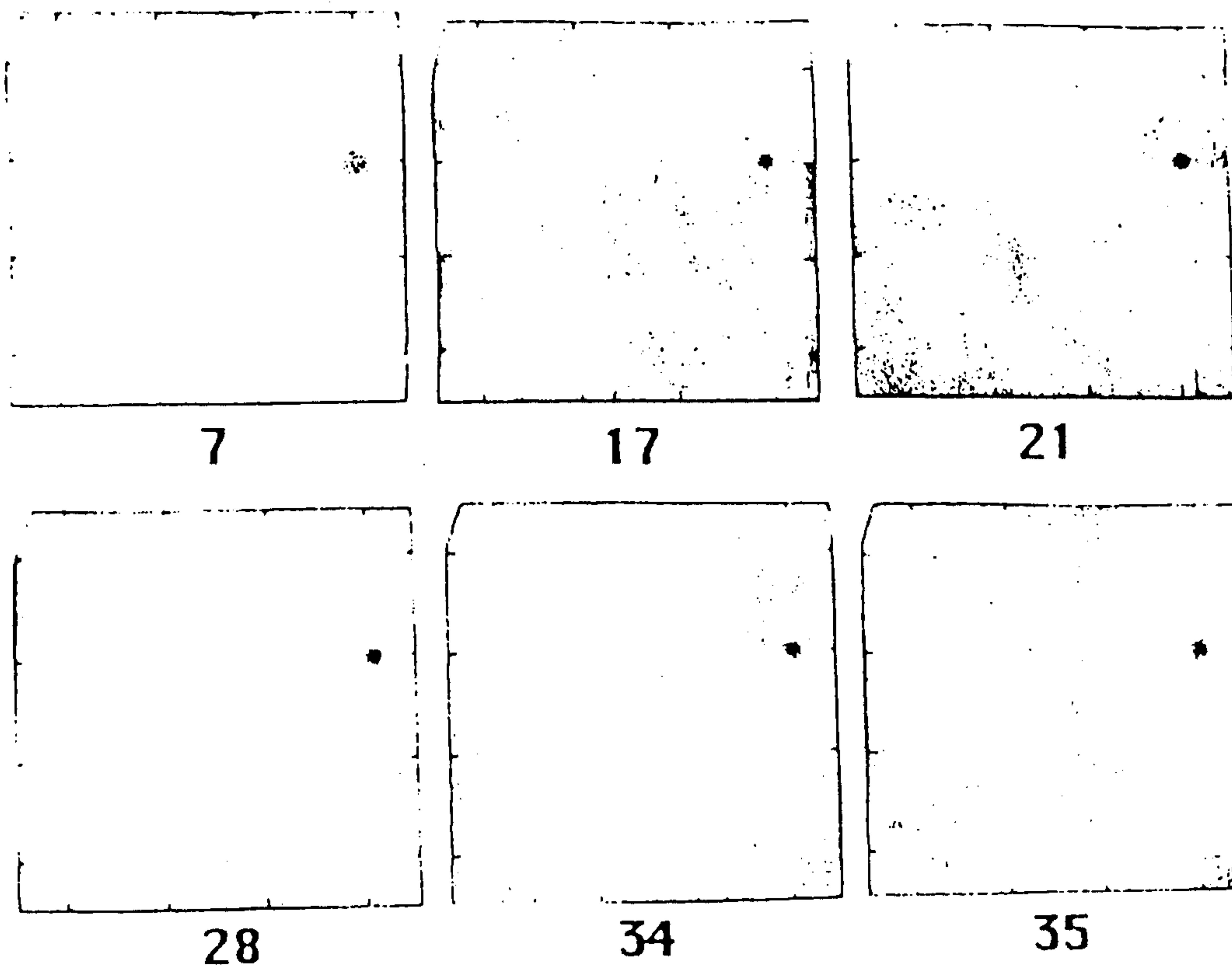
washing twice for 20 minutes at 55°C in an aqueous solution comprising 0.1 X SSC and 0.1% SDS.

Fig. 1

SA	SE	EC	KP
EBC	EF	PA	BF
BT	BU	HI	HPA
SP	SAG	SPN	HUM

SA:*S.aureus* /SE:*S.epidermidis* /EC:*E.coli* /KP:*K.pneumoniae* /
EBC:*E.cloacae* /EF:*E.faecalis* /PA:*P.aeruginosa* /BF:*B .fragilis* /
BT: *B.thetaiotaomicron* /BU:*B.vulgatus* /HI:*H.influenzae* /HPA:
H.parainfluenzae /SP:*S.pyogenes* /SAG:*S.agalactiae* /SPN:
S.pneumoniae /HUM: U937 genomic DNA

(a)



(b)