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(54) Titre : PROCEDE ET ACIDES NUCLEIQUES SERVANT A DETERMINER LA PRESENCE DE MICRO-ORGANISMES PRESENTANT UN INTERET DANS LE DOMAINE DE LA BRASSERIE
(54) Title: METHOD AND NUCLEIC ACIDS FOR DETERMINING THE PRESENCE OF MICRO-ORGANISMS SPECIFIC TO THE BREWING PROCESS

(57) **Abrégé/Abstract:**

The invention relates to a method for determining the presence of micro-organisms specific to the brewing process, in addition to nucleic acids and combinations thereof which can be used in said method. The invention also relates to the use of the inventive nucleic acids or combinations thereof for determining and/or identifying and/or classifying different genera or species of micro-organisms specific to the brewing process. The invention aims to provide a method and a means of rapidly testing beer and brewing raw materials for contamination by micro-organisms, whereby the test should encompass the whole range of possible beer-contaminative micro-organisms. This is achieved by a method which comprises the following steps: (a) the sample is brought into contact with a combination of at least two first nucleic acid molecules (primers) which hybridise with a conserved area of the microbial nucleic acid in micro-organisms specific to the brewing process; (b) amplification of the microbial nucleic acid or a portion thereof, in order to create at least one amplification fragment; (c) the amplification fragment obtained in step (b) is brought into contact with at least a second nucleic acid molecule (probe) which specifically hybridises with at least one amplification fragment comprising a sequence of the microbial nucleic acid specific to all the micro-organisms present in the brewing process, or specific to one or more families, genera or species of said micro-organisms; and (d) determining the presence of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule, introduced in step (c). The invention also relates to nucleic acid molecules which can be used according to the invention in said method.

Summary

The invention relates to a method for the detection of microorganism relevant to brewing, as well as to nucleic acids and combinations thereof which can be used in this method. The invention further relates to the use of the nucleic acids according to the invention or combinations thereof for the detection and/or for the identification and/or characterisation of different genera or species of microorganisms relevant to brewing. Hence the problem to be solved by the present invention was to provide a method and means which make possible a rapid test of beer and brewing raw materials for contamination with micro-organisms, the test being required to detect the whole range of possible beer-contaminating microorganisms. This problem is solved according to the invention by a method which comprises the following steps: (a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing; (b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment; (c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing; and (d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c). Further, nucleic acids are provided, which can be used in the method according to the invention.



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Method and Nucleic Acids for the Detection of Microorganisms Relevant to Brewing

The invention relates to a method for the detection of microorganisms relevant to brewing, as well as to nucleic acids and combinations thereof which can be used in this method. The invention further relates to the use of the nucleic acids according to the invention or combinations thereof for the detection and/or for the identification and/or characterisation of different genera or species of microorganisms relevant to brewing.

Beer can be regarded as very stable microbiologically, and can only be spoilt by a relatively manageable number of bacteria. In order to discover contamination with these organisms as early as possible, an analytical system which allows rapid detection of the microorganisms in the matrix beer must be used, since countermeasures must be undertaken immediately.

The common feature of all microorganisms harmful to beer is the trace contamination of individual vessels (barrels, bottles) and their slow growth. In particular, microbiological culturing of the anaerobic microorganisms is very difficult. The beer-spoiling bacteria at present known are classed into the following genera: *Lactobacillus*, *Pediococcus*, *Pectinatus* and *Megasphaera*. Members of the *Selenomonas* and *Zymophilus* genera have not yet emerged as beer contaminants, however contamination of beer and their subsequent growth in it cannot be ruled out.

The genus *Lactobacillus* describes Gram positive, non-sporulating, mostly immotile and chain-forming rods, which are long, thin and sometimes curved. Coccoid forms are also sometimes observed. Members of the genus *Lactobacillus* are microaerophilic, and some are anaerobic. They are cytochrome- and catalase-negative, their metabolism is fermentative and they require a complex nutrient medium. The molar G+C content of the DNA is between 32 and 53%.

As well as in beer, *Lactobacilli* are found in dairy and cereal products, in meat and fish products, in water, waste water, wine, fruit and fruit juices, acid-pickled vegetables, sauerkraut, silage and sourdough. Although they are a component of the normal oral, intestinal and vaginal flora of mammals, they are however seldom pathogenic (*Bergeys Manual of Syst. Microbiology*, 1984, p. 1209-1234). In beer, because of their metabolic

products, they lead to clouding and undesired flavour changes. Species relevant to beer spoilage are *Lactobacillus brevis*, *Lactobacillus lindneri*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus coryniformis* and *Lactobacillus curvatus* (Back, Brauwelt, 1980, 120, p. 1562-1569).

The genus *Pediococcus* includes Gram positive, immotile and non-sporulating cocci. They form tetrads or occur as pairs. They are facultative anaerobes, and their oxygen sensitivity differs from species to species. *Pediococci* are cytochrome and catalase-negative and require a complex nutrient medium (*Bergeys Manual of Syst. Microbiology*, 1984, p. 1075-1079). They are used as starter cultures for the production of raw sausage products, they ferment various types of pickled vegetables and lead to the spoilage of foodstuffs (Firnhaber, Baumgart: *Mikrobiologische Untersuchung von Lebensmitteln*, 1993, p. 413-419, 115-117). The genus includes 8 species, and the species *Pediococcus damnosus* and *Pediococcus inopinatus* should be regarded as harmful to beer.

The genus *Pectinatus* includes the species *Pectinatus cerevisiiphilus*, *Pectinatus frisingiensis* and the strain *Pectinatus* sp. DSM 20764, not further taxonomically classified. All strains have been isolated from spoilt beer (Schleifer et al., *Int. J. of Syst. Bacteriology*, 1990, p. 19-27). These are slightly bent, non-sporulating rod-shaped bacteria. They have comb-like flagella, and are motile. They produce neither catalase nor cytochrome oxidase, and are obligate anaerobes. The molar G+C content is 38-41%. In the genus *Pectinatus*, and also in the genera *Megasphaera*, *Selenomonas* and *Zymophilus*, the cell wall is more similar to the Gram positive bacteria than to the Gram negative bacteria. Although the Gram staining is negative, they are taxonomically classified among the Gram positive bacteria (Haikara, *The Prokaryotes*, 2nd Edition, Vol. II, 1991, p. 1993-2004).

The genus *Megasphaera* includes the species *Megasphaera elsdenii* and *Megasphaera cerevisiae*. Only *Megasphaera cerevisiae* is relevant to brewing, and is described as a Gram negative, strictly anaerobic, cytochrome- and catalase-negative, immotile and sometimes slightly stretched coccus, which occurs singly, in pairs or in short chains. The mean cell diameter is about 1.4 μm , and the molar G+C content 42.4-44.8%. Main metabolites are sulphur compounds such as H_2S and volatile fatty acids. In beer,

contamination with *Megasphaera cerevisiae* leads to very marked changes in aroma and taste (Haikara, *The Prokaryotes*, 2nd Edition, Vol. II, 1991, p. 1993-2004).

Species of the genus *Selenomonas* are defined as obligate anaerobes, Gram negative, non-sporulating, slightly curved and motile rods. The molar G+C content is about 48-58% (Schleifer et al., *Int. J. of Syst. Bacteriology*, 1990, p. 19-27). *Selenomonads* are isolated from the stomach and intestinal tract and the dung of mammals. The genus includes 10 species (Hespell et al., *The Prokaryotes*, 2nd Edition, Vol. II, 1991, p. 2005-2013). Only *Selenomonas lactificex* has been isolated from starter yeast, and is thus relevant to brewing. *Selenomonas lactificex* has not yet emerged as a beer-spoiling bacterium, however its growth in beer is possible, and hence it fulfils the definition of a beer-spoiling organism.

The species *Zymophilus paucivorans* and *raffinivorans* belong to the genus *Zymophilus* as Gram-negative, slightly bent, motile rods, which occur singly, in pairs or in short chains. The molar G+C content is about 38-41% . They are obligate anaerobes and have a fermentative metabolism. Both species are isolated from starter yeasts and brewery wastes; growth in beer has only been observed with *Zymophilus raffinivorans* (Schleifer et al., *Int. J. of Syst. Bacteriology*, 1990, p. 19-27).

On the basis of comparison of the 16S rRNA gene sequences, all the genera to be tested are classified among the Gram positive bacteria with low G+C content. The genera *Pediococcus* and *Lactobacillus* are classified into the *Lactobacillaceae* family, and the genera *Pectinatus*, *Megasphaera*, *Selenomonas* and *Zymophilus* into the *Sporomusa* group. The *Sporomusa* group is also described as a group of the Gram positive Eubacteriales with Gram-negative cell wall (Stackebrandt et al., *The Prokaryotes*, 2nd Edition, Vol. II, 1991, p. 25-26, 33).

A classical microbiological determination of the microorganisms described above can require up to 10 days. However, a markedly faster analysis is desirable, as otherwise unnecessary storage costs arise or the beer being tested has already been delivered. For these reasons, several rapid detection methods have already been developed. Thus for example organisms harmful to beer can be detected on the basis of their metabolic products (Haikara et al. *Microbiology*, 1995, 141, p. 1131-1137). Other indirect methods are turbidometry (Haikara et al., *ASBC*, 1990, p. 92-95) and measurement of the ATP

bioluminescence (Miller et al., *J. Inst. Brew.*, 1989, Vol. 95, p. 317-319). Detection by means of antibodies is also rapid and specific (Gares et al., *ASBC*, 1993, p. 158-163; Winnewisser et al., *Int. J. of Bacteriology*, 1995, 45, p. 403-405). With these methods, the disadvantage is that either non-specific parameters are tested or only one species or genus is detected in each case. Also, the equipment and staff cost is high. An overview of rapid methods for the detection of contaminants relevant to brewing is given by Dowhanick (*Cerevisia*, 1995, 20/4, p. 40-49).

The polymerase chain reaction (PCR; Mullis et al., see US 4,683,195, US 4,683,202 and US 4,965,188) is a rapid and effective method of specifically detecting organisms. A range of nucleic acids are known, through the use of which as primers and/or probes the specific detection of microorganisms relevant to brewing is possible. However, a disadvantage is that with the use of these nucleic acid molecules in an amplification or detection reaction, it is always only possible to detect a fraction of all possible microorganisms relevant to brewing. These PCR systems serve for the specific detection in each case only of individual species in an amplification reaction of the genera *Lactobacillus*, *Pediococcus*, *Pectinatus* and *Mega-sphaera* (Sakamoto US 5,869,642; Nietupski et al., US 5,705,339 and US 5,484,900; Tsuchia et al., JP 06141899A, JP 06113888A / *ASBC J.*, 1992, p. 64-67 / *ASBC J.*, 1993, p. 40-41; Yasui JP07289295A / *Can. J. Microbiol.*, 1997, 43, p. 157-163, Shimada et al., JP06090793; Alatossava et al. WO97/09448; Doyle et al., *J. of Ind. Microbiology*, 1995, 15, p. 67-70; DiMichele et al., *ASBC J.*, 1993, p. 63-66; Vogeser et al, *Brauwelt*, 1998, 24/25, p. 1060-1063). Further, the methods described for visualisation of the amplification products, such as for example agarose gel electrophoresis, present problems, as the carcinogenic and highly toxic ethidium bromide is used for staining the amplification products. These methods can only be automated with difficulty and the assessment of the agarose gels or the identification of the microorganisms on the basis of the length of the amplification products is sometimes not clear.

The problem to be solved by the present invention was therefore to provide a method and means which make possible a rapid test of beer and brewing raw materials for contamination with microorganisms, the test being required to detect the whole range of possible beer-contaminating microorganisms.

This problem is solved according to the invention by a process which comprises the following steps:

- (a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing;
- (b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment;
- (c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing;
- (d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c),

and by a nucleic acid molecule selected from:

- (i) a nucleic acid with a sequence according to SEQ ID NO 1-107 or a fragment thereof at least 10, preferably 15-30 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70%, preferably at least 90%, identical with a nucleic acid according to (i) or (ii), or
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

In the sequences according to SEQ ID NO 1-107, nucleotides are abbreviated as follows:

G = guanosine, A = adenosine, T = thymidine, C = cytidine, U = uracil, i = inosine. In accordance with IUPAC, mixtures are abbreviated as follows: R = G or A, Y = C or T, K = G or T, W = A or T, S = C or G, M = A or C, B = C, G or T, D = A, G or T, H = A, C or T, V = A, C or G, and N = A, C, G or T.

For the determination of identity (in the sense of complete agreement, corresponding to 100% identity) with nucleic acid sequences according to (iii), partial sequences of a larger polynucleotide are considered. These partial sequences include 10 nucleotides and are identical when all 10 building blocks are identical in the two sequences compared. The nucleotides thymidine and uridine are to be regarded as identical. All possible fragments of a larger polynucleotide can be regarded as partial sequences.

Here 90% identity is present, when in the two sequences to be compared 9 out of 10 or 18 out of 20 nucleotides in one section are identical.

As an example, let us consider two polynucleotides which comprise 20 nucleotides and differ in the 5th element. In a sequence comparison, six 10-nucleotide ones are then found which are identical, and 5 which are not identical, as they differ in one element.

Otherwise, the identity can also be determined by degree, the unit being stated in percent. For determination of the degree of identity, partial sequences are also considered, which as a minimum include the length of the sequence actually used, e.g. as primer, or else 20 nucleotides.

As an example, polynucleotides A with a length of 100 nucleotides and B with a length of 200 nucleotides are compared. From polynucleotide B, a primer with a length of 14 nucleotides is derived. For the determination of the degree of identity, polynucleotide A is compared with the primer over its whole length. If the sequence of the primer occurs in polynucleotide A, but differs in one element, then there is a fragment with a degree of identity of $13/14 \rightarrow 92.3\%$.

In the second example, the whole of the aforesaid polynucleotides A and B are compared. In this case, all possible comparison windows of a length of 20 nucleotides are applied, and the degree of identity determined for them. Thus if nucleotides 50-69 of polynucleotide A and B are identical with the exception of nucleotide No. 55, then for these fragments a degree of identity of $19/20 \rightarrow 95\%$ is found.

The method according to the invention can be carried out more rapidly than the previous microbiological detection methods, and makes it possible to detect several, preferably all, microorganisms relevant to brewing potentially present in a sample, such as for example even *Lactobacillus* species or members of the genera *Selenomonas* or *Zymophilus*

seldom arising as contaminants, for which hitherto no detection method existed. The detection is comprehensive and indicates all contamination risks in the brewery. By means of the method according to the invention, microorganisms relevant to brewing can be detected both in beer samples and also in raw material samples (barley malt, yeast, hops, water) or samples of intermediate products in beer production (e.g. mash, wort) even when the number of contaminating microorganisms is still low.

In this context, microorganisms relevant to brewing are understood primarily to mean bacteria and in particular the bacteria described above *Lactobacillus brevis*, *Lactobacillus lindneri*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus coryniformis*, *Lactobacillus curvatus*, *Pediococcus damnosus*, *Pediococcus inopinatus*, *Pectinatus cerevisii-philus*, *Pectinatus frisingiensis*, *Pectinatus* sp. DSM 20764, *Megasphaera cerevisiae*, *Selenomonas lacticifex*, *Zymophilus paucivorans* and *Zymophilus raffinivorans*, and also all microorganisms to be found in beer, which while they do not belong to the aforesaid species, can nonetheless multiply in beer, for example rare members of the *Lactobacillaceae* family such as *Lactobacillus malefermentans*, *Lactobacillus buchneri*, *Lactobacillus parabuchneri*, *Lactobacillus sanfrancisco*, *Lactobacillus delbrueckii*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus* and *Lactococcus lactis*.

The microorganisms detectable by the method according to the invention are thus not limited to the microorganisms hitherto described as beer contaminants. Rather, the use of the nucleic acid molecules and the method according to the invention offers the possibility of recognising the presence of other microorganisms relevant to brewing, which have not previously been described as beer contaminants. A positive result at the level of higher taxonomic units (e.g. orders, families, genera) combined with a negative result at the level of the lower taxonomic units known to be relevant to brewing (e.g. species, subspecies, strains) indicates a contamination with such a non-typical microorganism relevant to brewing.

In a first step of the method according to the invention, the sample to be tested is brought into contact with a combination of at least two first nucleic acid molecules (primers). These nucleic acid molecules hybridise with a region of a microbial nucleic acid which is conserved in microorganisms relevant to brewing. The hybridisation takes place through pairing of the primer with regions of the microbial nucleic acid which have an at least

partly complement-ary base sequence. The term "conserved" characterises the evolutionary variability of nucleotide sequences for species of different taxonomic units. If corresponding sequence sections from at least two microorganisms relevant to brewing are compared, the sequence can be regarded as variable or as conserved. Comparison sequences which are at least 95% identical are described as conserved, and those which are less than 95% identical as variable. Thus a region of a nucleic acid conserved in microorganisms relevant to brewing denotes a region which is at least 95% identical in all microorganisms relevant to brewing (as defined above).

In a preferred embodiment of the present invention, the conserved region occurs in a genome section which contains the bacterial 23S and 5S genes. This region includes the intergenic spacer between the genes for the 23S rRNA and the 5S rRNA and the bounding 23S and 5S rDNA genes, and includes both conserved sequence regions and also hypervariable (i.e. very organism-specific) sequence regions. Prokaryotic ribosomes as a rule contain three distinct nucleic acid components, which are generally known as 5S, 16S and 23S rRNA (ribosomal nucleic acid). The genetic information for these ribonucleic acids (rDNA) is typically arranged in the genome as a tandem. The typical organisation of such a unit is 16S-23S-5S, where the genes are connected to one another by short hypervariable intergenic regions, so-called spacers. The units are present several times in the genome, and the number of operons can vary from species to species. The high conservation of the DNA sequence in certain sections of the ribosomal DNA over the whole bacterial kingdom allows the design of non-specific oligonucleotides even without exact knowledge of the individual DNA sequences of the organisms to be investigated. The sequences according to SEQ ID NO 1-20 according to the invention (Table 1) are sequences of the 23S-5S intergenic spacer of microorganisms relevant to brewing, from which nucleic acid molecules for use in the method according to the invention can be derived.

The combination of at least two first nucleic acid molecules used in the first step of the method according to the invention is selected such that they are usable as primers in an amplification reaction, i.e. one nucleic acid molecule hybridises onto a first conserved region of the first strand of the target DNA and the other nucleic acid onto a second conserved region of the DNA strand complementary to the first, wherein the desired target region of the DNA is included. Both nucleic acid molecules have a length of at

least 10 bp, preferably 15-30 bp. In a preferred embodiment of the invention, a combination of at least two nucleic acid molecules according to this invention is used. In a particularly preferred embodiment of the invention, a combination is used which includes at least one nucleic acid molecule with a sequence according to one of the SEQ ID NO 40 to 47 (Table 2) and at least one nucleic acid molecule with a sequence according to SEQ ID NO 48-54 or SEQ ID NO 55-59 or SEQ ID NO 60-72 (Table 2).

In a second step of the method according to the invention, the microbial nucleic acid or a portion thereof is amplified, whereby at least one amplification fragment is produced. Amplification is understood to mean the raising of the concentration of a nucleic acid or a portion thereof present in a reaction mixture. Processes used for the amplification of nucleic acids are for example the PCR (US 4,683,195, US 4,683,202 and US 4,965,188), the "self-sustained sequence replication" (EP 329,822), the "transcription-based amplification system" (EP 310,229) and the " β -RNA replicase system" (US 4,956,858). In a preferred embodiment of the present invention, the amplification comprises a polymerase chain reaction (PCR). In a further embodiment of the present invention, the amplification comprises a ligase-chain reaction or an isothermal nucleic acid amplification.

In a third step of the method according to the present invention, the amplification fragments obtained are brought into contact with at least one second nucleic acid molecule (probe). This nucleic acid molecule or these nucleic acid molecules hybridise specifically with at least one amplification fragment that comprises a sequence of the microbial nucleic acid which is specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing, i.e. only occurs in members of these families or genera or in these species.

The double-strand formation of two identical or similar nucleotide fragments (DNA, RNA, PNA) is described as hybridisation. The term specific hybridisation is used when a stable hybrid nucleic acid between the oligonucleotide and the corresponding target DNA of the oligonucleotide exists, but not to other DNA than the target DNA. For the purposes of this invention, the feature "sequence which specifically hybridises with a sequence according to (i)" refers to a sequence which under stringent conditions hybridises with the sequence according to (i). For example, the hybridisations can be carried out at 50°C with a hybridisation solution consisting of 2.5 x SSC, 2 x Denhardt's solution, 10 mM Tris,

1 mM EDTA pH 7.5. Suitable washing conditions are for example four times repeated 1-minute washings in 0.1 x SSC to 1.0 x SSC, 2 x Denhardts, 10 mM Tris, 1 mM EDTA, pH 7.5 at 20-50°C.

In a preferred embodiment of the invention, one or several of the nucleic acid molecules according to the invention is used as a second nucleic acid molecule (probe).

Consensus probe is understood to mean a nucleic acid molecule which hybridises with highly conserved regions of a microbial nucleic acid and reacts with the amplification products of all microorganisms relevant to brewing. Nucleic acid molecules according to the invention which are usable as consensus probes have a sequence according to one of SEQ ID NO 40 to 72 (Table 2).

For the detection of a specific genus of microorganisms relevant to brewing, a nucleic acid molecule with a sequence according to one of SEQ ID NO 35 to 39 or SEQ ID NO 104 to 107 (Table 2) is preferably used. The genus specificity of a probe is defined as the ability of this probe to hybridise with the DNA of all isolates of as large as possible a group of members of the particular genus to be detected.

Species-specific nucleic acid probes are understood to mean nucleic acid molecules which hybridise with the DNA of all isolates of the particular species to be detected under the same stringency conditions. Species-specific nucleic acid molecules according to the invention with SEQ ID NO 21-22, SEQ ID NO 25-34, SEQ ID NO 73-78, SEQ ID NO 80-85 or SEQ ID NO 87-97 (Table 2) can be used.

The probes SEQ ID NO 23-24, SEQ ID NO 79, SEQ ID NO 86 and SEQ ID NO 98 to 103 are special cases. With the probes according to SEQ ID NO 23 and SEQ ID NO 79, strains of *Lactobacillus casei* and *Lactobacillus paracasei* ssp. *paracasei* can be detected. A probe according to SEQ ID NO 24 allows the detection of two subspecies of *Lactobacillus coryniformis* (*L. coryniformis* ssp. *coryniformis* and *L. coryniformis* ssp. *torquens*). With the probe SEQ ID NO 86, strains of the species *Pediococcus damnosus*, *Pediococcus inopinatus* and *Pediococcus parvulus* can be detected. With the use of these probes, other microorganisms relevant to brewing are not detected. Likewise, with the probes SEQ ID NO 98 to 103, all species of the *Lactobacillaceae* family relevant to brewing to be detected are detected, and other species and genera relevant to brewing are discriminated against.

In the last step of the method according to the invention, the detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in the preceding step takes place.

Preferably, first nucleic acid molecules (primers) and/or second nucleic acid molecules (probes) are at least 10 nucleotides, preferably 15-30 nucleotides long. In one embodiment of the present invention, the first and/or the second nucleic acid molecules are modified in that up to 20% of the nucleotides in 10 consecutive nucleotides, in particular 1 or 2 nucleotides of a block of 10 are replaced by nucleotides which do not occur naturally in bacteria.

The method according to the invention preferably includes the so-called consensus PCR. In this method, multiplication of the microbial nucleic acid or a portion thereof, and subsequent detection of these molecules by hybridisation with labelled specific probes take place. In the consensus PCR, nucleic acid molecules are used which make it possible to obtain an amplification product from several or indeed all the relevant strains, subspecies, species or genera. The amplification does not lead to a differentiation of the microorganisms. The specificity of the detection is achieved through the subsequent hybridisation reaction with specific probes. In this way, microorganisms relevant to brewing can be simultaneously detected in a simple combination of amplification and detection reaction.

This kind of amplification and detection makes it possible to automate the detection reaction, so that a high sample throughput becomes possible. For example, a PCR-ELISA detection procedure can be used, in which the respective probes are bound in different wells of a microtitre plate, in which the hybridisation and the detection of the labelled amplification products then occurs. The detection can also be effected by the use of a microarray, on which several probes are immobilised, as a result of which the detection reaction can be carried out quickly and at no great cost.

In a preferred embodiment of the invention, the second nucleic acid molecule (probe) is modified or labelled in such a way that it can produce a detectable signal. The modification or labelling is selected from (i) radioactive groups, (ii) coloured groups, (iii) fluorescent groups, (iv) groups for immobilisation on a solid phase and (v) groups

which permit an indirect or direct reaction, especially with the aid of antibodies, antigens, enzymes and/or substrates with affinity to enzymes or enzyme complexes.

For the purposes of this invention, labelling indicates directly or indirectly detectable groups or groups for immobilisation on a solid phase, which are attached to the nucleic acid molecule. Directly detectable are metal atoms, radioactive, coloured or fluorescent groups. Indirectly detectable are immunologically or enzymatically detectable groups, for example antigens and antibodies, haptens or enzymes or enzymatically active parts of enzymes. These indirect groups are detected in subsequent reactions. Preferred are haptens which are coupled to an oligonucleotide and which are detected in a subsequent antibody reaction.

The nucleic acid molecules according to the invention can be used for the detection and/or for the identification and/or characterisation of bacteria relevant to brewing. The primers and/or probes described herein can also be used in the detection of the described microorganisms in drinks other than beer, in other samples from the brewing sector, such as for example in raw materials, starter yeast, environmental samples, in other foodstuff samples or in clinical samples, etc.

Examples:

Example 1: Determination of the DNA target sequence of the bacteria harmful to beer and closely related species

By sequence comparison of known 23S rDNA and 5S rDNA sequences (GenBank Sequence Database of the National Center of Biotechnology Information: NCBI), conserved gene regions were identified, which serve as hybridisation sites for the primers used for the sequencing. From pure cultures of the bacteria listed in Table 1, genomic DNA was isolated by known standard methods. With primers which hybridise in highly conserved regions, amplification products of all bacteria to be detected were obtained in a PCR. The following primers were used for the amplification and the subsequent sequencing:

Primer 1 = SEQ ID NO 47:

5'-AAG TGC TGA AAG CAT CTA AG-3'

Primer 2 = SEQ ID NO 55:

5'-GGC RRY GTC TAY TYT CSC-3'

Composition of the PCR:

Genomic DNA (10 – 100 ng)	1.00 μ l	
H ₂ O	16.85 μ l	
Buffer (10 x)	2.50 μ l	1 x
dNTP (10 mM)	0.50 μ l	200 μ M
Primer 1 = Seq ID NO 48 (5 μ M)	1.50 μ l	0.30 μ M
Primer 2 = Seq ID NO 49 (5 μ M)	1.50 μ l	0.30 μ M
MgCl ₂ (50 mM)	1.00 μ l	2.00 mM
Taq-polymerase (5 U/ μ l)	0.15 μ l	0.03 U/ μ l
Σ	25.00 μ l	

Temperature profile:

5 mins	95°C	
30 secs	95°C	
30 secs	50°C	x 38
30 secs	72°C	
5 mins	72°C	

These amplification products were purified via an agarose gel and by a subsequent treatment with the QIAquick PCR Gel Extraction Kit (Quiagen Co.) and sequenced in the Long Read Sequencer Model 4000L (LI-COR Co.) with the aforesaid primers, which are provided with an IRD-800 label. The resulting sequences of the 23S/5S rDNA spacer regions of the bacteria relevant to brewing and the phylogenetically closely related species were compared with one another and sequence regions identified which:

- 1.) are to be found in all species of the particular genus to be detected and at the same time differ from those of other genera or species,
- 2.) are only to be found in the particular species to be detected, but differ from other bacteria to be detected and not to be detected.

In the sequence regions described under 1.), hybridisation sites of genus-specific oligonucleotides were defined, and in the sequence regions described under 2.), the binding sites of species-specific oligonucleotides were defined.

Example 2: Detection of Bacteria Harmful to Beer by the Polymerase Chain Reaction

I. Amplification

Genomic DNA was isolated from pure cultures of the bacteria listed in Table 1 by known standard methods. Decimal dilutions from 1 fg/ μ l to 1 pg/ μ l of these preparations were then used in a PCR with the following composition:

Primer 3 = SEQ ID NO 46:

5'-AAG GGC CAT CRC TCA ACG G -3'

Primer 4 = SEQ ID NO 48:

5'-TGT GTT CGI iAT GGG AAC AGG TG -3'

Genomic DNA	1.00 μ l	4.00 μ l	
H ₂ O	16.60 μ l	66.40 μ l	
Buffer (10 x)	2.50 μ l	10.00 μ l	1 x
dNTP (10 mM)	0.50 μ l	2.00 μ l	0.20 mM
Primer 3 = Seq ID NO 21 (5 μ M)	1.50 μ l	6.00 μ l	0.30 mM
Primer 4 = Seq ID NO 22 (5 μ M)	1.50 μ l	6.00 μ l	0.30 mM
digoxigenin labelled			
DMSO (100%)	0.25 μ l	1.00 μ l	1.00 %
MgCl ₂ (50 mM)	1.00 μ l	4.00 μ l	2.00 mM
Taq-polymerase (5 U/ μ l)	0.15 μ l	0.60 μ l	0.03 U/ μ l
Σ	25.00 μ l	100.00 μ l	

The PCR was performed under the following conditions in the Mastercycler® (Eppendorf Co.) according to the following temperature profile:

5 mins	95°C	x 38
30 secs	95°C	
45 secs	55°C	
90 secs	72°C	
5 mins	72°C	

Primer 3 (SEQ ID NO 46) was determined by sequence comparison of known 23S rDNA sequences (GenBank Sequence Database of NCBI). It hybridises onto highly conserved sequence sections in the 23S rDNA gene region. The binding site lies outside the region sequenced with the primers SEQ ID NO 48 and 49.

Primer 4 (SEQ ID NO 48) was determined on the basis of our own sequence data. The hybridisation site of primer 2 lies adjacent to the intergenic 23S/5S spacer in the 5S rDNA region.

II. Detection by PCR-ELISA

The detection is effected by PCR-ELISA. For this, per probe used, 5 µl of amplification product are treated with 5 µl of denaturation buffer (125 mM NaOH, 20 mM EDTA, pH 14) and incubated for 15 mins at room temperature. Each time, 2 pmoles of the particular biotinylated probe are pipetted into 100 µl of hybridisation buffer (2.5 x SSC, 2 x Denhardts solution, 10 mM Tris, 1 mM EDTA, pH 7.5) and transferred to the wells of a microtitre plate coated with streptavidin and preincubated at the hybridisation temperature of 50°C. After the denaturation, the denaturation mixture is pipetted into the hybridisation mixture. Next the mixture is incubated for 30 minutes at hybridisation temperature. If the hybridisation is complete, the hybridisation mixture is removed and the plate washed 4x with 200 µl of wash buffer 1 (WB1: 0.1 x SSC, 2 x Denhardts, 10 mM Tris, 1 mM EDTA, pH 7.6) for 1 min. each time at hybridisation temperature. Next, 100 µl of a solution of a horseradish peroxidase conjugated anti-digoxigenin antibody diluted according to the manufacturer's instructions is added (Boehringer Mannheim). The conjugate is diluted in wash buffer 2 (WB2: 100 mM Tris, 150 mM NaCl, 0.05% Tween 20, 0.5% blocking reagent, 100 µg/ml herring sperm, pH 7.6). Next, the antibody incubation is performed at 37°C for 30 mins. After this, the plate is washed four times with 200 µl of WB2 (at room temperature). After the washing, 100 µl of POD

substrate (Boehringer Mannheim) are added and the mixture incubated for 20 mins at RT. Next the colour reaction is stopped with 100 μ l of 0.5M H_2SO_4 and estimated at 450 nm.

III. Assessment

According to the detection protocol described above, the detection was performed for all bacteria and bacteria groups investigated, using the corresponding genus- and species-specific probes. Genus-specific probes are SEQ ID NO 35 for *Pediococcus*, SEQ ID NO 36 for *Pectinatus*, SEQ ID NO 37 for *Megasphaera*, SEQ ID NO 38 for *Selenomonas* and SEQ ID NO 39 for *Zymophilus*. Species-specific probes are SEQ ID NO 21 for *Lactobacillus brevis*, SEQ ID NO 22 for *Lactobacillus lindneri*, SEQ ID NO 23 for *Lactobacillus casei* + *paracasei*, SEQ ID NO 24 for *Lactobacillus coryniformis*, SEQ ID NO 25 for *Lactobacillus curvatus*, SEQ ID NO 26 for *Pediococcus damnosus*, SEQ ID NO 27 for *Pediococcus inopinatus*, SEQ ID NO 28 for *Pectinatus cervisiiphilus*, SEQ ID NO 29 for *Pectinatus frisingiensis*, SEQ ID NO 30 for *Pectinatus* sp. DSM20764, SEQ ID NO 31 for *Megasphaera cerevisiae*, SEQ ID NO 32 for *Selenomonas lacticifex*, SEQ ID NO 33 for *Zymophilus paucivorans* and SEQ ID NO 34 for *Zymophilus raffinivorans*.

As controls, the consensus probes SEQ ID NO 40 and 41 were used, which hybridise with the amplification products of all the species to be detected. Further possible binding sites for consensus probes are SEQ ID NO 42-45. The probes of SEQ ID NO 40 to 45 were determined by sequence comparison of known 23S rDNA and 5S rDNA sequences (GenBank Sequence Database, NCBI).

If the extinction measured for a 1 fg quantity of genomic DNA used in the PCR was greater than 1, the result was assessed as positive. The results of the PCR-ELISA are presented in Table 3.

Table 1

SEQ ID NO	Source			Description	Sequence	
	Genus	Species	Strain			
1	Lactobacillus	brevis	DSM 20054	23S-spacer-5S	5'-TATATGGAAG TAAGACCCCT GAGAGATGAT CAGTAGATA GGCTGGAAGT AGCAGCGCCG TGAGGCGTGG AGCGGACCAG TACTAATCGG TCGAGGACTT AACCAAGTCA ACAACGTAAGT TGTTTCGAGA ATAATTGAAT AATATCTAGT TTTGAGGGAA GAAGTTCTCT TATAGTGTGG TGGCGATAGC CTGAAGGATA CACCTGTTCC CATGCCGAAC ACAGAAGTTA AGCTTCAGCA CGCCGATAGT AGTTGGGGA TCGCCCC-3'	50 100 150 200 250
2	Lactobacillus	lindneri	DSM 20690	23S-spacer-5S	5'-CCATTCCCTAT ATGGAAGTAA GACTCCTGAA AGATGATCAG GTCGATAGGT TAGAAGTGA AGCATAGTGA TATGTGAAGC GGACTAATAC TAATCAGTCG AGGACTTAAC CAAGGAAGAC ACAGGTTAA ATCAAAAGTTG AACAGAGAAG ATATTATCTA GTTTTGAGAG AACGAAGTTC GCTCAGGCTT ATGAAAAATA AGCATAGTGT GGTGGCGATA GCCTGAAGGA TACACCTGTT CCCATGCCGA ACACAGAAGT TAAGCTTCAG CACGCCAAA GTAGTTGGG GATCGCCCC TGCAGGATA GGACGATGTT CATAGC-3'	50 100 150 200 250 300
3	Lactobacillus	casei	DSM 20011	23S-spacer-5S	5'-CCATTCCCTAT ATGGAAGTAA GACCCCTGAG AGATGATCAG GTAGATAGGC TGGAAGTGA AGTGCAGCGA TGCATGGAGC GGACCAGTAC TAATCGGTCG AGGACTTAAC CAAGTAGAGC GTAGCAGGA GCGCTTAGAA ACCGGAGCAT AAGCGGGCCT GAGTTCGTTG GCCGGGTTT GGCCAAATGA TTCAGGGTTC TTATGTGGAG GTTCTGCGA CTGCGAACGC GTTTCGATGA AATACACTGG TTCCCGACAA CACAAAACA ACAATGATAG CCAGTTTGA GAGCGCAAAG TTCTCATAAG TGTGGTGGCG ATAGCAAGAA GGATACACCT GTTCCCATGC C-3'	50 100 150 200 250 300 350
4	Lactobacillus	paracasei ssp. paracasei	DSM 20008	23S-spacer-5S operon 1	5'-CCATTCCCTAT ATGGAAGTAA GACCCCTGAG AGATGATCAG GTAGATAGGC TGGAAGTGA AGTGCAGCGA TGCATGGAGC GGACCAGTAC TAATCGGTCG AGGACTTAAC CAAGTAAGAG TGTGAGCAGG AGCGGTTAGA AACCGGAGCA TAAGCGGGCC TGAGCGTGAT GGCCGGGCTT TGGCCATTGC GGTACAGGTC CTTATGTGCA GGTTCCTGCG ACTGCGAACA CGTTTCGATG ACAAGTACGT TAAGTTCAAG GCAGCAATTA AACAAATGATA GCTAGTTTGG AGAGCGCAAA GTTCTCATAA GTGTGGTGGC GATAGCAAGA AGGATACACC TGTTCCCATG CCGAACACAG AAGTTAAGCT TCTTCACGCC GAGAGTAGTT GTTGGGAAAC TGCCCTGCGAG GATA-3'	50 100 150 200 250 300 350 400

SEQ ID NO	Source		Description	Sequence	
5	Lactobacillus	paracasei ssp. paracasei	DSM 20008	5'- 23S-spacer-5S operon 2	CCATTCTCTAT ATGGAAGTAA GACCCCTGAG AGATGATCAG GTAGATAGGC 50 TGGAAAGTGA AGTGCAGCGA TGCATGGAGC GGACCAGTAC TAATCGGTCG 100 AGGACTTAAC CAAGTAAGCG TGCAAGCAGG AGCAGGTTTC TCGCACTGCG 150 AACACATTTT GATGACAAAGT ACGTTAAGTT CAAGGCAGCA ATTAACAGAT 200 GATAGCCAGT TTTGAGAGCG CAAAGTTCTC ATAAAGTGTG TGGCGATAGC 250 AAGAAGGATA CACCTGTTCC CATGCCGAAC ACAGAAAGTTA AGCTTCTTCA 300 CGCCGAGAGT AGTTGGTGGG AAACCTGCCCTG CGAGGATA-3'
6	Lactobacillus	coryniformis ssp. coryniformis	DSM 20001	5'- 23S-spacer-5S	CTCGAGTTGA GATTTCCTCAT TCCTTTATGG AAGTAAGACC CCTGAGAGAT 50 GATCAGGTAG ATAGGTTGGA AGTGGACGTG CCGTGAGGCA TGGAGCGGAC 100 CAATACTAAT CGGTCGAGGA CTTAACCAGG TAGCATGTAC GTAGTGTTAG 150 TTTAAGGGCA AAGAAATGAA TATCCAGTTT TGAGAGCGCA ACGTTCTCAG 200 AAAGTGGTGT GGTGGCGATA GCAAGAAGGA TACACCTGTT CCATGTGCGA 250 ACACAGAAGT TAAGCTTCTT AGCGCCGAGA GTAGTTGGGG GAGCACCCCC 300 TGGGAGGATA GGACGAT-3'
7	Lactobacillus	coryniformis ssp. torquens	DSM 20004	5'- 23S-spacer-5S	CTCGAGATGA GATTTCCTCAT TCCTTTATGG AAGTAAGACC CCTGAGAGAT 50 GATCAGGTAG ATAGGTTGGA AGTGGACGTG CCGTGAGGCA TGGAGCGGAC 100 CAATACTAAT CGGTCGAGGA CTTAACCAGG TAGCATGTAC GTGGTGTAG 150 TTTAAGGGCA AAGAAATGAA TATCCAGTTT TGAGAGCGCA ACGTTCTCAG 200 AAAGTGGTGT GGTGGCGATA GCAAGAAGGA TACACCTGTT CCATGTGCGA 250 ACACAGAAGT TAAGCTTCTT AGCGCCGAGA GTAGTTGGGG GAGCACCCCC 300 TGGGAGGATA GGACGAT-3'
8	Lactobacillus	curvatus	DSM 20019	5'- 23S-spacer-5S	ACGCCTCGAG ATGAGATTTC CCATTCTTAT ATGGAAGTAA GACCCCTGAA 50 AGATGATCAG GTAGATAGGC TAGGAGTGA AGTACAGCGA TGTATGGAGC 100 GGACTAGTAC TAATCGGTCG AGGACTTAAC CAAAGGTGCA ATGTTAGGCT 150 TTTGAAATGA AATATTACTT ATTATGCAGT TTTGAGAGAA CGAAGTTCTT 200 CTCAGTGCGC AAGCACAAA TAGTGTGGTG GCGATAGCAA GAAGGATACA 250 CCTGTTCCCA TGTCGAACAC AGAAGTTAAG CTTCTTAGCG CCGATAGTAG 300 TTGGTGGGAA ACTACCTGCG AGGATAGGAC GATGGT-3'
9	Pediococcus	damnosus	DSM 20331	5'- 23S-spacer-5S	GATGAGATTT CCCATTCCAT TTATGGAAGT AAGACCCCTG AGAGATGATC 50 AGGTAGATAG GTTGGGAGTG GAAAGTGTAGT GATACATGGA GCGGACCAAT 100 ACTAATCGGT CGAGGACTTA ACCACAAAAGT GGTGTTCTCA AGAGAAGGAT 150 TCGATATTAT TTAGTTTGA GAGAATAAAT TTCTTTTACA CGAGCCCGCT 200 AAGTGGATCG GAGAAGTGTG GTGACGATAG TGAGAAGGAT ACACCTGTTC 250 CCATGTGCGA CACAGAAGTT AAGCTTCTTA ACGCCGAGAG TAGTTGGGG 300 ATCGCTCCCT GCGAGGATAG GACGATGGTC AATAG-3'

SEQ ID NO	Source		Description	Sequence	
10	Pediococcus	inopinatus	DSM 20285	5'-	AGATGAGATT TCCCATTTCCA TTTATGGAAG TAAGACCCCT GAGAGATGAT CAGGTAGATA GGTGGGAGT GGAAGTGAG TGATACATGG AGCGGACCAA TACTAATCGG TCGAGGACTT AACACAAAG TGGTGTCTC AAAGAGAAGA TTTCGATATT ATTTAGTTTT GAGAGAATAA ATTTCTTTCA CACGAGCCGC GGAAGTGGAT CGGAGAAAGT TGGTGACGAT AGTGAGAAGG ATACACCTGT TCCCATGTGG AACACAGAAG TTAAGCTTCT TAACGCCGAG AGTAGTTGGG GGATCGCTCC CTGCGAGGAT AGGACG-3'
11	Pectinatus	cerevisiiphilus	DSM 20467	5'-	AAGTGCTGAA AGCATCTAAG CGTGAAACCT GCCTTAAGAT GAGGTTTCCC AGAGCCGTAA GGCTTGGAAG GCACCTTGAA TAAGACGAGG TAGATAGGCC GGGAGTAGAA GTACAGTAAT GTACGAAGCG GACTGGTACT AATAAGCCGA GAGCTTAAC TAAATCATC GAAAAAATG TTTGGTCTGA GATTCTTCT GTGAAGTTTT GAGTGTGCAA GACACTCTGG TTGAAGGGCA GGGAACGTGA GAGCGTAAA CTGCGGACTT TGGCTCAAAG AGTTAAAGCA TCTGGTGACG ATACCTGGAT GGATCCACCT GTTCCCATTC CGAACACAGT AGTTAAGCAT CCACAGGCTG AAGTACTTG GGGGCGGACC CCCTGGGAAA ATAGGACACT GCC-3'
12	Pectinatus	frisingensis	DSM 6306	5'-	AAGTGCTGAA AGCATCTAAG CGTGAAACCA GCTTTAAGAT GAGGTTTCCC AGAACGCAAG TTTGGAAGGC ACCTTGAAGA AGACGAGGTA GATAGGCCGG GAGTGGAAGT ATGGTGACAT ATGAAGCGGA CTGGTACTAA TAAGCCGAGA GCTTAACTTG ATTTTCATCA AAAAGAGAAA TGTTTGGTCA GAGATTTTCT TCTGTGAAGT TTTGAGTGTG CAAGAACACT CGAGAGTATA TAGGTAAGG AAAAGCAGCA GATAAGTTTC CTGGTTACTG TATATACCGG CTGAGGTGCT GAGGCACTGA AGGCCAGAAC ATCTGGTGGC GATACCTGGA TGGATCCACC TGTTCCTCAT CCGAACACAG TAGTTAAGCA TCCACAGGCC GAAGGTACTT GGGGGCAGC CCCCTGCGAA AATAGGACAC CGCC-3'

SEQ ID NO	Source		Description	Sequence	
13	Pectinatus	sp.	DSM 20764	23S-spacer-5S operon 1	5'- AAGTGCTGAA AGCATCTAAG CGTGAAACCT GCCTTAAGAT GAGGTTTCCC 50 AGAGCCGTAA GGCTTGAAG GCACCTTGAA GATGACGAGG TAGATAGGCC 100 GGGAGTAGAA GTATGGTGAC ATACGAAGCG GACTGGTACT AATAAGCCGA 150 GAGCTTAACT TAATTTCATC TATAAATGTT TGGTCCTGAT TTCTTCTGTG 200 AAGTTTGTAG TGTGCAAGAT CACTCATGAA AGTATATAGG TAAAGGGAAA 250 GCAGCAGATT AGTTCCTGGT TTACTTTATA TAAGAGCACT AAGGTGCAGA 300 AAAGAACGTT TGAGGAAACG CGGCGTTCGT AAACCTCACTT TGCCTGCTGA 350 TTATCTCAAT GCTAAAGCAT TAAGATAATT TTAGAGGAAA CGCGCGTTCA 400 CTAGCGTTCA CTCTGCGTAC TTTATTTCTA AGTGCTGAAG CACTAAGAAG 450 GGCAAGGAAA CGCGTCGTC GCGATGCTCA CTTTGCGTAC TTCATCTCTA 500 GACTGCTAAA GCAGTAAGAT CTGAAGCATC TGGTGGCGAT ACCTGGATGG 550 ATCCACCTGT TCCCATTCGG AACACAGTAG TTAAGCATCC ACAGGCCGAA 600 GGTACTTGGG GGCAGCCCC CTGCGAGAGT AGGACATCGC C-3'
14	Pectinatus	sp.	DSM 20764	23S-spacer-5S operon 2	5'- AAGTGCTGAA AGCATCTAAG CGTGAAACCT GCCTTAAGAT GAGGTTTCCC 50 AGAGCCGTAA GGCTTGAAG GCACCTTGAA GATGACGAGG TAGATAGGCC 100 GGGAGTAGAA GTATGGTGAC ATACGAAGCG GACTGGTACT AATAAGCCGA 150 GAGCTTAACT TAATTTCATC TATAAATGTT TGGTCCTGAT TTCTTCTGTG 200 AAGTTTGTAG TGTGCAAGAT CACTCATGAA AGTATATAGG TAAAGGGAAA 250 GCAGATTAGT TCCTGGTTTA CTTTATATAT GAGCACTAAG GTGCAGAAAA 300 GAACGCTCTAA GGAACGCGG CGTTGCTAGG CTCACCTCTG GTACTTTCATC 350 TCTAGACTGC TAAAGCAGTA AGATCTGAAG CATCTGGTGG CGATACCTGG 400 ATGGATCCAC CTGTTCCCAT TCCGAACACA GTAGTTAAGC ATCCACAGGC 450 CGAAGGTACT TGGGGGCGAG CCCCTGCGA AAGTAGGACA CCGCC-3'
15	Megasphaera	cerevisiae	DSM 20462	23S-spacer-5S operon 1	5'- GCATCTAAGC GTGAAACCAG CCTAGAGATG AGGTTTCTCA TTACGAAAGT 50 AAGTAAGGTC CCATGAAGAC GACATGGTAG ATAGGCCGGG AGTGGACGTA 100 CAGTAATGTA TGGAGCGGAC CGGTACTAAT AGACCGAGGA CTTGACTTAA 150 GCAGGGAACC CATTTTAAAG AAGCGAAGCG ACGCATAAAA TGGAGTGAGT 200 CGCTTATACC GAATCGCAGA TTCGGTAAAG CAGCGGAGAA TACCAATGCA 250 GCGGCAACAC CAGTTAGCAT AAACCTAAGCG GATTCCGGAGT GGGTGAGGGA 300 GTTTCGTAGC AGCGTAGGCT AACCCAAACA CCGCTTTCGA AGAAGGCCGAA 350 TGGTTTGAAG AAGAGTACAT GCGAAGAAAC GACGAAAGAC TCACAACCAA 400 AACATACAAA CTAAGTAGAT GACATTAGAG TCACACCCGAT TGTAAAGATC 450 CGAAATACTT TTCGATGTAG TTGTCAGGAT ACGAATCCTG AAACGAATTC 500 AGTGGTGATG GCTGCAGGGA TCCACCTGTT CCCATACCGA ACACAG-3'

SEQ ID NO	Source		Description	Sequence	
16	Megasphaera	cerevisiae	DSM 20462	5'-	GCATCTAACC GTGAAACCAG CCTAGAGATG AGGTTTCTCA TTACGAAAGT
				23S-spacer-5S operon 2	50 AAGTAAGGTC CCATGAAGAC GACATGGTAG ATAGGCCGGG AGTGGACGTA 100 CAGTAATGTA TGGAGCGGAC CGGTACTAAT AGACCGAGGA CTTGACTTAA 150 GCAAGAAGC AATAGAAAGA ACCATGTAGA TGGTGTAAGA GTTAGACGGG 200 TAGTTAAGGT CCGAAATACT TTTTCGATGTA GTTGTACAGG TACGAATCCT 250 GAAACGAATT CAGTGGTGAT GGCTGCAGGG ACCACCTGTT CCCTATACCGA 300 ACACAG-3'
17	Selenomonas	lactificifex	DSM 20757	5'-	AAGTGCTGAA AGCATCTAGG CGTGAAGCCT GTCCCCGAGAT GAAGTATCTC
				23S-spacer-5S operon 1	50 ATGGAGTAAT CCAGTAAGAT TCCTTGAAGA AGACAAGGTA GATAGGTTGG 100 GAGTGTAAGC ATCGTAAGGT GTTCAGCGGA CCAATACTAA TAAATCGAGG 150 GCTTAACCTT ACAGACCTGT CCAAGAAGCG AAGCGGATTG GGTAAACAGGT 200 CGTATGCGAA AACATCCCAA GAATCGAGTC CGAAGGGCGA AGATGATTGG 250 CAGATGTTGA CCGCTAATAA TCTAGAATGT TTCGATACAA TTTTCTTCTT 300 GTATAGTTT GAGTGGACAT CGTTCATTCA ATAATATCCA GTGACGATAG 350 CTGAGTGGTA CCACCTGTTT CCATACCGAA CACAGTAGTT AAGCACTCAT 400 ACGCCGAAAG TACTTGTCTG GAAACGGGCT GCGAGAATAG GACGTGCGCC -3'
18	Selenomonas	lactificifex	DSM 20757	5'-	AAGTGCTGAA AGCATCTAAG CGTGAAGCCT GTCCCCGAGAT GAAGTATCTC
				23S-spacer-5S operon 2	50 ATGGAGTAAT CCAGTAAGAT TCCTTGAAGA AGACAAGGTA GATAGGTTGG 100 GAGTGTAAGC ATCGTAAGGT GTTCAGCGGA CCAATACTAA TAAATCGAGG 150 GCTTATCTTA ATAATCTAGA ATGTTTCGAT ACAATTTTC TTCTGTATAG 200 TTTGTAGTGG ACATGGTTCA TTCAATAATA TCCAGTGACG ATAGCTGAGT 250 GGTACCACCT GTTCCCATAC CGAACACAGT AGTTAAGCAC TCATACGCCG 300 AAAGTACTTG TCTGGAAACG GGCTGCGAAA ATAGGACGCC GCC-3'
19	Zymophilus	raffinivorans	DSM 20765	5'-	AAGTGCTGAA AGCATCTAAG CGTGAACCA GCCTTAAGAT GAGGTTTCTC
				23S-spacer-5S	50 ACAGAGCAAT CTGGTAAGAC CCTTGAAGA AGACAAGGTA GATAGGTCGG 100 GAGTGAAGC GCAGTAATGT GTGCAGCGGA CCGATACTAA TAGGTCGAGG 150 GCTTGACTTA AAGCCAGAAC GAAACTAAA ATGCGAACAT TTCCTTCTTC 200 TGTATAGTTT TGAGAGAACA AACTCTTAAG ATGGAGTAGT CTGAGCGGAA 250 AGCGGAAGGC AGCGATATCT AAAAAAGAA TATCTGGTAG TGATAGCCAA 300 GTGGACCCAC CTGTTCCCAT ACCGAACACA GTAGTTAAGC ACTTGAACGT 350 CGAAAGTACT TGGTGGAAA CGCCCTGCCA AAATAGGACA CCGCC-3'

SEQ ID NO	Source		Description	Sequence	
	Zymophilus	paucivorans			
20			23S-spacer-5S	5'-AAGTGCTGAA AGCATCTAAG CGTGAAACCA GCCTTAAGAT GAGGTTTCTC ACAGAGCAAT CTGGTAAGAC CCCTTGAAGA AGACAAGGTA GATAGGTCGG GAGTGGAAGC GCAGTAATGT GTGTAGCGGA CCGATACTAA TAGGTCGAGG GCTTGACTTA AAGCCAGAAC GAATCTTAA ATCGAACAT TTCTTTCTTC TGTATAGTTT TGAGAGAACA GACTCTTAAG ATGAGCAGTC TGAGGCGAAA GCTAAAGGCA GCGATATCTA AAAAAAGAA TATCTGGTAG TGATAGCCAA GTGGACCCAC CTGTTCCCAT ACCGAACACA GTAGTTAAGC ACTGAACGT CGAAAGTACT TGGGTGAAA CGCCCTGGGA AAATAGGACA CCGCC-3'	50 100 150 200 250 300 350

Table 2

SEQ ID NO	Description			Sequence	
21	Lactobacillus brevis	Specific probe	5'-	CCAAGTCAACAACGTAGTTGT	-3'
22	Lactobacillus lindneri	Specific probe	5'-	GACACAGGGTTAAATCAAAGTTG	-3'
23	Lactobacillus casei Lactobacillus paracasei ssp. paracasei	Specific probe	5'-	AGGTTTCTGCGACTGCGAAC	-3'
24	Lactobacillus coryniformis ssp. coryniformis Lactobacillus coryniformis ssp. torquens	Specific probe	5'-	ATGTACGTAGTGTAGTTTAAGGGC	-3'
25	Lactobacillus curvatus	Specific probe	5'-	CTTCTCAGTGCAGCACACA	-3'
26	Pediococcus damnosus	Specific probe	5'-	GTGTTCTCAAGAGAGAGATTTCG	-3'
27	Pediococcus inopinatus	Specific probe	5'-	GTTCTCAAAGAGAGAAGATTTCGATATTA	-3'
28	Pectinatus cerevisiiphilus	Specific probe	5'-	TGAGAGCGTAATAACTGCGGACTT	-3'
29	Pectinatus frisingensis	Specific probe	5'-	CAGATAAGTTTCCCTGTTACTG	-3'
30	Pectinatus sp. DSM 20764	Specific probe	5'-	CACTAAGGTGCAGAAAAGAACGT	-3'
31	Megasphaera cerevisiae	Specific probe	5'-	CTTTTCGATGTAGTTGTCAGGATACG	-3'
32	Selenomonas lacticifex	Specific probe	5'-	GTTTCATTCAATAATATCCAGTGACG	-3'
33	Zymophilus raffinosivorans	Specific probe	5'-	AACTCTTAAGATGGAGYAGTCTG	-3'
34	Zymophilus paucivorans	Specific probe	5'-	ACTCTTAAGATGAGCAGTCTGA	-3'
35	Pediococcus genus	genus-specific probe	5'-	AGTSTAGTGATACATGGAGCG	-3'
36	Pectinatus genus	genus-specific probe	5'-	GTGAAGTTTTTGAGTGTGCAAGA	-3'
37	Megasphaera genus	genus-specific probe	5'-	GACCGAGGACTTGACTTAAGCA	-3'
38	Selenomonas genus	genus-specific probe	5'-	TCCAGTGACGATAGCTGAGT	-3'
39	Zymophilus genus	genus-specific probe	5'-	AAGAATATCTGGTAGTGATAGCCAA	-3'

Table 2 (Cont.)

SEQ ID NO	Description		Sequence	
40	consensus sequence	5'-	GTCGTGAGACAGTTCGGTC	-3'
41	consensus sequence	5'-	CYTAGTACGAGAGGACCGRR	-3'
42	consensus sequence	5'-	GCTACCCCTGGGGATAACAGGC	-3'
43	consensus sequence	5'-	ATCGACGGGGAGGTTTSSCAC	-3'
44	consensus sequence	5'-	CACCTCGAATGTCGGCTCRTC	-3'
45	consensus sequence	5'-	CCAAGGGTTGGGCTGTTC	-3'
46	consensus sequence	5'-	AAGGGCCATCRCTCAACGG	-3'
47	consensus sequence	5'-	AAGTGCTGAAAGCATCTAAG	-3'
48	consensus sequence	5'-	TGTGTTGCTGATGGGAACAGGTG	-3'
49	consensus sequence	5'-	TGTGTTGCGAATGGGAACAGGTG	-3'
50	consensus sequence	5'-	TGTGTTGCGAAATGGGAACAGGTG	-3'
51	consensus sequence	5'-	TGTGTTGCGGTATGGGAACAGGTG	-3'
52	consensus sequence	5'-	TGTGTTGCGATATGGGAACAGGTG	-3'
53	consensus sequence	5'-	TGTGTTGCGCATGGGAACAGGTG	-3'
54	consensus sequence	5'-	TGTGTTGCGACATGGGAACAGGTG	-3'
55	consensus sequence	5'-	GGCRRYGTCTCTAITYTTCSC	-3'
56	consensus sequence	5'-	GGCAGTGTCTCTACTTTCCC	-3'
57	consensus sequence	5'-	GGCAGCGTCTCTACTTTCCG	-3'
58	consensus sequence	5'-	GGCAGTGTCTCTACTTTCCG	-3'
59	consensus sequence	5'-	GGCAGCGTCTCTACTTTCCC	-3'
60	consensus sequence	5'-	GYTMRCTTCYRDGTTTCG	-3'
61	consensus sequence	5'-	GCTTAACTTCCGTTTCG	-3'
62	consensus sequence	5'-	GCTTAACTTCTATGTTTCG	-3'
63	consensus sequence	5'-	GCTTAACTTCTGTTTCG	-3'
64	consensus sequence	5'-	GCTTAACTTCCATGTTTCG	-3'
65	consensus sequence	5'-	GCTTAACTTCCGGGTTTCG	-3'
66	consensus sequence	5'-	GCTTAACTTCTAGGTTTCG	-3'
67	consensus sequence	5'-	GCTTAACTTCTGGGTTTCG	-3'
68	consensus sequence	5'-	GCTTAACTTCCAGGTTTCG	-3'
69	consensus sequence	5'-	GCTTAACTTCCGAGTTTCG	-3'
70	consensus sequence	5'-	GCTTAACTTCTAAGTTTCG	-3'

Table 2 (Cont.)

SEQ ID NO	Description			Sequence	
71	consensus sequence		5'-	GCTTAACTTCTGAGTTCG	-3'
72	consensus sequence		5'-	GCTTAACTTCCAAGTTCG	-3'
73	Lactobacillus	brevis	specific probe	TCGAGAATAATTGAATAATATCTAG	-3'
74	Lactobacillus	brevis	specific probe	GAGGGAAGAAGTTCCTCTTAT	-3'
75	Lactobacillus	lindneri	specific probe	AACAGAGAAGATATATCTAGTT	-3'
76	Lactobacillus	lindneri	specific probe	TTGAGAGAACGAAGTTCGCTCAGGCTTATGAAAAATAAGCAT	-3'
77	Lactobacillus	casei	specific probe	TTCGTTGGCCGGGTTTGGCCCAATGGATTTCAGGGTTCCTTATGTGG	-3'
78	Lactobacillus	casei	specific probe	GCGTTTCGATGAATAACACTGTTTCCCGACACACAAAAACAATGATAGCCAGTT	-3'
79	Lactobacillus	casei	specific probe	TTAGAAACCGGAGCATAAAGCGGCCTGAG	-3'
80	Lactobacillus	paracasei	specific probe	GCGTGATGGCCGGGCTTTGGCCCATTCGGGTCAAGGTCCTTATGTGC	-3'
81	Lactobacillus	paracasei	specific probe	CAAGTACGTTAAGTTCAAGGCAGCAATTAACAATGATAGTAGTT	-3'
82	Lactobacillus	coryniformis	specific probe	AAAGAAATGAATATCCAGTTTTCAGAGCGCAACGTTCTCAGAAA	-3'
83	Lactobacillus	curvatus	specific probe	AGGTGCAATGTAGGCTTTTGAATGAAATATATTAATTTATATGCAGTT	-3'
84	Pediococcus	damnosus	specific probe	GCCGCGTAAGTGGATCGGAGAA	-3'
85	Pediococcus	inopinatus	specific probe	GCCGCGGAAGTGGATCGGAGAA	-3'
86	Pediococcus	inopinatus	specific probe	GAGAGAATAAATTTCTTTTCACACGA	-3'
87	Pectinatus	cerevisiophilus	specific probe	AAAATCATCGAAAAAATGTTTGGTCTGAGATTTCTTCT	-3'
88	Pectinatus	cerevisiophilus	specific probe	CACTCTGTTGAAGGCAGGGAACG	-3'
89	Pectinatus	frisingensis	specific probe	GATTTTCATCAAAAAAGAGAAATGTTTGGTCAGAGATTTT	-3'
90	Pectinatus	frisingensis	specific probe	TATATACCGGCTGAGGTGCTGAGGCACTGAAGG	-3'
91	Pectinatus	sp. DSM 20764	specific probe	AATTTTCATCTATAAATGTTTGGTCCCTGATTTCTTCT	-3'
92	Pectinatus	sp. DSM 20764	specific probe	AGATTAGTTCTGTTTACTTTATATATGAGCACTAAGGTGCAGAAAAG AACGT	-3'
93	Pectinatus	sp. DSM 20764	specific probe	AGGAAACGCGGCTTCGTAA	-3'
94	Selenomonas	lactificex	specific probe	TAATAATCTAGAATGTTTCGATACAAATTTTCTTCTGTATAGTTTGTAG TGACAT	-3'
95	Zymophilus	raffinivorans	specific probe	GAGCGGAAAGCGGAAGGCAGCGAT	-3'
96	Zymophilus	paucivorans	specific probe	GAGCGGAAAGCTAAAGGCAGCGAT	-3'
97	Megasphaera	cerevisiae	specific probe	AATCCTGAACGAATTCAGTGGTGATGGCTGCAGGGA	-3'

Table 2 (Cont.)

SEQ ID NO	Description		Sequence	
98	Detection of all Lactobacillaceae relevant to brewing for differentiation from other bacteria relevant to brewing	5'-	TATGGAAGTAAGACCCCTGA	-3'
99		5'-	AGATGATCAGGTAGATAGGCT	-3'
100		5'-	AGATGATCAGGTCGATAGGTT	-3'
101		5'-	AGATGATCAGGTAGATAGGTT	-3'
102		5'-	TACTAATCGGTCGAGGACTTAACCA	-3'
103		5'-	ATACTAATCAGTCGAGGACTTAACCA	-3'
104		5'-	GAAGCGGACTGGTACTAATAAGCCGAGAGCTT	-3'
105	Pectinatus	5'-	CAGCGGACCAATACTAATAAATCGAGGGCTTA	-3'
106	Selenomonas	5'-	AGCGGACCGATACTAATAAGTCGAGGGCTTGACTTAAA	-3'
107	Zymophilus	5'-	GGAGCGGACCCGGTACTAATAAGACCGAGGACTT	-3'
	Megasphaera	5'-		-3'

Table 3

[illegible]

	SEQ ID NO 33	SEQ ID NO 34	SEQ ID NO 35	SEQ ID NO 36	SEQ ID NO 37	SEQ ID NO 38	SEQ ID NO 39	SEQ ID NO 40-45
<i>Lactobacillus brevis</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus lindneri</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus casei</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus paracasei paracasei</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus coryniformis coryniformis</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus coryniformis torquens</i>	-	-	-	-	-	-	-	+
<i>Lactobacillus curvatus</i>	-	-	-	-	-	-	-	+
<i>Pediococcus damnosus</i>	-	-	+	-	-	-	-	+
<i>Pediococcus inopinatus</i>	-	-	+	-	-	-	-	+
<i>Pectinatus cerevisiiphilus</i>	-	-	-	+	-	-	-	+
<i>Pectinatus frisingensis</i>	-	-	-	+	-	-	-	+
<i>Pectinatus sp. DSM 20462</i>	-	-	-	+	-	-	-	+
<i>Megasphaera cerevisiae</i>	-	-	-	-	+	-	-	+
<i>Selenomonas lacticifex</i>	-	-	-	-	-	+	-	+
<i>Zymophilus raffinivorans</i>	+	-	-	-	-	-	+	+
<i>Zymophilus paucivorans</i>	-	+	-	-	-	-	+	+

SEQUENCE LISTING

<110> BioteCon Diagnostics GmbH

<120> Method and nucleic acids for the detection of microorganisms relevant to brewing

<130> PCT1218-066

<140>

<141>

<160> 107

<170> PatentIn Ver. 2.1

<210> 1

<211> 267

<212> DNA

<213> Lactobacillus brevis

<400> 1

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tgtttcgaga ataattgaat aatatctagt tttagaggaa gaagttctct tatagtgtgg 180
tggcgatagc ctgaaggata cacctgttcc catgccgaac acagaagtta agcttcagca 240
cgccgatagt agttggggga tcgcccc 267
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<210> 2

<211> 326

<212> DNA

<213> Lactobacillus lindneri

<400> 2

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agcatagtga tatgtgaagc ggactaatac taatcagtcg aggacttaac caaggaagac 120
acagggttaa atcaaagttg aacagagaag atattatcta gttttgagag aacgaagttc 180
gctcaggctt atgaaaaata agcatagtgt ggtggcgata gcctgaagga tacacctgtt 240
cccatgccga acacagaagt taagcttcag cagccaaaa gtagttgggg gatcgcccc 300
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<210> 3

<211> 351

<212> DNA

<213> Lactobacillus casei

<400> 3

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gtgagcagga gcgcttagaa accggagcat aagcgggcct gagttcgttg gccgggtttt 180
ggccaatgga ttcagggttc ttatgtggag gtttctgcga ctgcgaacgc gtttcgatga 240
aatacactgg ttcccgaaca caaaaaaca acaatgatag ccagttttga gagcgcaaag 300
ttctcataag tgtggtggcg atagcaagaa ggatacacct gttcccatgc c 351
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<210> 4
 <211> 414
 <212> DNA
 <213> *Lactobacillus paracasei*

<400> 4
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 tgtgagcagg agcgggttaga aaccggagca taagcgggcc tgagcgtgat ggccgggctt 180
 tggccattgc ggtcaggggc cttatgtgca ggtttctgcg actgcgaaca cgtttctgatg 240
 acaagtacgt taagttcaag gcagcaatta aacaatgata gctagttttg agagcgcaaa 300
 gttctcataa gtgtgggtggc gatagcaaga aggatacacc tgttcccatg ccgaacacag 360
 aagttaagct tcttcacgcc gagagtagtt ggtgggaaac tgctgagcag gata 414

<210> 5
 <211> 338
 <212> DNA
 <213> *Lactobacillus paracasei*

<400> 5
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 tgcaagcagg agcaggtttc tgcgactgcg aacacatttc gatgacaagt acgttaagtt 180
 caaggcagca attaaacgat gatagccagt tttgagagcg caaagttctc ataagtgtgg 240
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 cgccgagagt agttgggtggg aaactgcctg cgaggata 338

<210> 6
 <211> 317
 <212> DNA
 <213> *Lactobacillus coryniformis* ssp. *coryniformis*

<400> 6
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 ataggttgga agtggacgtg ccgtgaggca tggagcggac caataactaat cggtcgagga 120
 cttaccaag tagcatgtac gtagtggttag tttaagggca aagaaatgaa tatccagttt 180
 tgagagcgca acgttctcag aaagtgggtg ggtggcgata gcaagaagga tacacctgtt 240
 cccatgtcga acacagaagt taagcttctt agcgccgaga gtagttgggg gagcaccccc 300
 tgcgaggata ggacgat 317

<210> 7
 <211> 317
 <212> DNA
 <213> *Lactobacillus coryniformis* ssp. *torquens*

<400> 7
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 cttaccaag tagcatgtac gtgggtggttag tttaagggca aagaaatgaa tatccagttt 180
 tgagagcgca acgttctcag aaagtgggtg ggtggcgata gcaagaagga tacacctgtt 240
 cccatgtcga acacagaagt taagcttctt agcgccgaga gtagttgggg gagcaccccc 300
 tgcgaggata ggacgat 317

<210> 8
 <211> 336
 <212> DNA
 <213> *Lactobacillus curvatus*

<400> 8
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 tttgagagaa cgaagttctt ctcagtgcgc aagcacaaaa tagtgtggtg gcgatagcaa 240
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 ttggtgggaa actacctgcg aggataggac gatggt 336

<210> 9
 <211> 335
 <212> DNA
 <213> *Pediococcus damnosus*

<400> 9
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 gttgggagtg gaagtgtagt gatacatgga gcggaccaat actaatcggg cgaggactta 120
 accacaaagt ggtgttctca agagaaggat tcgatattat ttagtittga gagaataaat 180
 ttctttcaca cgagccgcgt aagtggatcg gagaagtgtg gtgacgatag tgagaaggat 240
 acacctgttc ccatgtcgaa cacagaagtt aagcttctta acgccgagag tagttggggg 300
 atcgctccct gcgaggatag gacgatgggc aatag 335

<210> 10
 <211> 326
 <212> DNA
 <213> *Pediococcus inopinatus*

<400> 10
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 aaccacaaag tgggtgttct aaagagaaga tttcgatatt atttagtttt gagagaataa 180
 atttctttca cagagccgc ggaagtggat cggagaagtg tggtgacgat agtgagaagg 240
 atacacctgt tcccatgtcg aacacagaag ttaagcttct taacgccgag agtagttggg 300
 ggatcgctcc ctgcgaggat aggacg 326

<210> 11
 <211> 403
 <212> DNA
 <213> *Pectinatus cerevisiiphilus*

<400> 11
 aagtgtgaa agcatctaag cgtgaaacct gccttaagat gaggtttccc agagccgtaa 60
 ggcttgggag gcacctgaa taagacgagg tagataggcc gggagtagaa gtacagtaat 120
 gtacgaagcg gactggtact aataagccga gagcttaact taaaatcatc gaaaaaatg 180
 tttggtctga gatttcttct gtgaagtttt gagtgtgcaa gacactctgg ttgaagggca 240
 gggaacgtga gagcgtaaaa ctgcggactt tggctcaaag agttaagca tctggtgacg 300
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 aaggtacttg gggggcgacc ccctgggaaa ataggacact gcc 403

<210> 12
 <211> 434
 <212> DNA
 <213> *Pectinatus frisingensis*

<400> 12
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 tttggaaggc accttgaaga agacgaggta gataggccgg gagtggaagt atggtgacat 120
 atgaagcgga ctggtactaa taagccgaga gcttaacttg atttcacaa aaaagagaaa 180
 tgtttggtca gagattttct tctgtgaagt tttgagtgtg caagaacact cgagagtata 240
 taggtaaagg aaaagcagca gataagtttc ctggttactg tatataccgg ctgaggtgct 300
 gaggcactga aggccagaac atctggtggc gatacctgga tggatccacc tgttcccatt 360
 ccgaacacag tagttaagca tccacaggcc gaaggtactt ggggggcagc cccctgcgaa 420
 aataggacac cgcc 434

<210> 13
 <211> 641
 <212> DNA
 <213> *Pectinatus spec. DSM20764*

<400> 13
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 ggcttggaag gcaccttgaa gatgacgagg tagataggcc gggagtagaa gtatggtgac 120
 atacgaagcg gactggtact aataagccga gagcttaact taatttcac tataaatggt 180
 tggctcctgat ttcttctgtg aagttttgag tgtgcaagat cactcatgaa agtatatagg 240
 taaagggaaa gcagcagatt agttcctggt ttactttata tatgagcact aaggtgcaga 300
 aaagaacggt tgaggaaacg cggcggttcgt aaactcactt tgcgtgctga ttatctcaat 360
 gctaaagcat taagataatt ttagaggaaa cgcgcgttca cttagcgttca ctctgcgtac 420
 tttattttcta agtgctgaag cactaagaag ggcaaggaaa cgcgtcgttc gcgatgctca 480
 ctttgcgtag ttcatctcta gactgctaaa gcagtaagat ctgaagcatc tgggtggcgat 540
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 ggtacttggg gggcagcccc ctgagagagt aggacatcgc c 641

<210> 14
 <211> 495
 <212> DNA
 <213> *Pectinatus spec. DSM20764*

<400> 14
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 atacgaagcg gactggtact aataagccga gagcttaact taatttcac tataaatggt 180
 tggctcctgat ttcttctgtg aagttttgag tgtgcaagat cactcatgaa agtatatagg 240
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 gaacgtctaa ggaaacgcgg cgttcgtagg ctactctgc gtacttcac tctagactgc 360
 taaagcagta agatctgaag catctggtgg cgatacctgg atggatccac ctgttcccat 420
 tccgaacaca gtagttaagc atccacaggc cgaaggtact tggggggcag cccctgcga 480
 aagtaggaca ccgc 495

<210> 15
 <211> 546
 <212> DNA
 <213> *Megasphaera cerevisiae*

<400> 15

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cgggtactaat agaccgagga cttgacttaa gcagggaacc catttttaaag aagcgaagcg 180
acgcataaaa tggagtgagt cgcttatacc gaatcgcaga ttcggtaaag cagcggagaa 240
taccaatgca gcggcaacac cagttagcat aaactaagcg gattcggagt gggtgaggga 300
gtttcgtagc agcgtaggct aacccaacca ccgctttcga agaaggcgaa tggtttgaaa 360
aagagtacat gcgaagaaac gacgaaagac tcacaaccaa aacatacaaa ctaagtagat 420
gacattagag tcacaccgat tgttaagatc cgaaatactt ttcgatgtag ttgtcaggat 480
acgaatcctg aaacgaattc agtggtgatg gctgcaggga tccacctgtt cccataccga 540
acacag                                         546

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<210> 16

<211> 306

<212> DNA

<213> *Megasphaera cerevisiae*

<400> 16

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cgggtactaat agaccgagga cttgacttaa gcaaagaagc aatagaaaga accatgtaga 180
tggtgtaaga gttagacggg tagttaaggt ccgaaatact tttcgatgta gttgtcagga 240
tacgaatcct gaaacgaatt cagtggatg ggctgcaggg accacctgtt cccataccga 300
acacag                                         306

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<210> 17

<211> 449

<212> DNA

<213> *Selenomonas lacticifex*

<400> 17

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ccagtaagat tccttgaaga agacaaggta gataggttgg gagtgtgaa atcgtaaggat 120
gttcagcggg ccaatactaa taaatcgagg gcttaacttt acagacctgt ccaagaagcg 180
aagcggattg ggtaacaggt cgtatgcgaa aacatcccaa gaatcgagtc cgaagggcga 240
agatgattgg cagatgttga ccgctaataa tctagaatgt ttcgatacga tttttcttct 300
gtatagtttt gagtggacat cgttcattca ataatatcca gtgacgatag ctgagtggta 360
ccacctgttc ccataccgaa cacagtagtt aagcactcat acgccgaaag tacttgtctg 420
gaaacgggct gcgagaatag gacgtcgcc                                         449

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<210> 18

<211> 343

<212> DNA

<213> *Selenomonas lacticifex*

<400> 18

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gttcagcggg ccaatactaa taaatcgagg gcttatctta ataactaga atgtttcgat 180
acaatttttc ttctgtatag ttttgagtgg acatggttca ttcaataata tccagtgcgc 240
atagctgagt ggtaccacct gttcccatac cgaacacagt agttaagcac tcatacgccg 300
aaagtacttg tctggaaacg ggctgcgaaa ataggacgcc gcc                                         343

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<210> 19
 <211> 395
 <212> DNA
 <213> *Zymophilus raffinosisivorans*

<400> 19
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 gtgcagcggg ccgatactaa taggtcgagg gcttgactta aagccagaac gaaaactaaa 180
 atgcgaacat ttctttcttc tgtatagttt tgagagaaca aactcttaag atggagtagt 240
 ctgaggcgaa agcggaaggc agcgatatct aaaaaaagaa tatctggtag tgatagccaa 300
 gtggacccac ctgttcccat accgaacaca gtagttaagc acttgaacgt cgaaagtact 360
 tgggtggaaa cgccctgcga aaataggaca ccgcc 395

<210> 20
 <211> 395
 <212> DNA
 <213> *Zymophilus paucivorans*

<400> 20
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 ctggtaagac cccttgaaga agacaaggta gataggtcgg gagtggaagc gcagtaatgt 120
 gtgtagcggg ccgatactaa taggtcgagg gcttgactta aagccagaac gaattctaaa 180
 atgcgaacat ttctttcttc tgtatagttt tgagagaaca gactcttaag atgagcagtc 240
 tgaggcgaaa gctaaaggca gcgatatcta aaaaaaagaa tatctggtag tgatagccaa 300
 gtggacccac ctgttcccat accgaacaca gtagttaagc acttgaacgt cgaaagtact 360
 tgggtggaaa cgccctggga aaataggaca ccgcc 395

<210> 21
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Description of the artificial sequence: specific
 sequence for *Lactobacillus brevis*

<400> 21
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<210> 22
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Description of the artificial sequence: specific
 sequence for *Lactobacillus lindneri*

<400> 22
 gacacagggt taaatcaaag ttg 23

<210> 23

<211> 20
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus casei and Lactobacillus
paracasei

<400> 23
aggtttctgc gactgcgaac

20

<210> 24
<211> 25
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus coryniformis

<400> 24
atgtacgtag tgtagttta agggc

25

<210> 25
<211> 20
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus curvatus

<400> 25
cttctcagtg cgcaagcaca

20

<210> 26
<211> 22
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Pediococcus damnosus

<400> 26
gtgttctcaa gagaaggatt cg

22

<210> 27
<211> 27
<212> DNA
<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pediococcus inopinatus*

<400> 27

gttctcaaag agaagatttc gatatta

27

<210> 28

<211> 23

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus cerevisiiphilus*

<400> 28

tgagagcgta aaactgcgga ctt

23

<210> 29

<211> 22

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus frisingensis*

<400> 29

cagataagtt tcctggttac tg

22

<210> 30

<211> 23

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus spec.* DSM 20764

<400> 30

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23

<210> 31

<211> 26

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Megasphaera cerevisiae*

<400> 31

cttttcgatg tagttgtcag gatacg

26

<210> 32

<211> 25

<212> DNA

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sequence for Selenomonas lacticifex

<400> 32

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25

<210> 33

<211> 23

<212> DNA

<213> Artifical sequence

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<223> Description of the artifical sequence: specific
sequence for Zymophilus raffinivorans

<400> 33

aactcttaag atggagyagt ctg

23

<210> 34

<211> 22

<212> DNA

<213> Artifical sequence

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sequence for Zymophilus paucivorans

<400> 34

actcttaaga tgagcagtct ga

22

<210> 35

<211> 21

<212> DNA

<213> Artifical sequence

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<223> Description of the artifical sequence: specific
sequence for the genus Pediococcus

<400> 35

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21

<210> 36

<211> 22

<212> DNA
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<223> Description of the artificial sequence: specific
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22

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<223> Description of the artificial sequence: specific
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22

<210> 38
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<223> Description of the artificial sequence: specific
 sequence for the genus Selenomonas

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<223> Description of the artificial sequence: specific
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 aagaatatct ggtagtgata gccaa

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<210> 40
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gtcgtgagac agttcggtc

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cytagtacga gaggaccggr r

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<210> 42
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21

<210> 43
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atcgacgggg aggtttssca c

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<210> 44
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<210> 45
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<223> Description of the artifical sequence: consensus sequence

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ccaaggggttg ggctgttc

18

<210> 46

<211> 19

<212> DNA

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aagggccatc rctcaacgg

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<210> 47

<211> 20

<212> DNA

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<223> Description of the artifical sequence: consensus sequence

<400> 47

aagtgtgtaa agcatctaag

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<210> 48

<211> 23

<212> DNA

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tgtgttcgii atgggaacag gtg

23

<210> 49

<211> 23

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tgtgttcgga atgggaacag gtg

23

<210> 50
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<210> 51
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<210> 53
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<210> 54
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tgtgttcgac atgggaacag gtg

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<210> 55

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<210> 56

<211> 19

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<210> 57

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<210> 58

<211> 19

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<400> 58

ggcagtggtcc tactttcgc

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<210> 59

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<400> 59

ggcagcgtcc tactttccc

19

<210> 60

<211> 18

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<210> 61

<211> 18

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<400> 61

gcttaacttc cgtgttcg

18

<210> 62

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<400> 62

gcttaacttc tatgttcg

18

<210> 63

<211> 18

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<400> 63

gcttaacttc tgtgttcg

18

<210> 64

<211> 18

<212> DNA
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<400> 64
gcttaacttc catgttcg 18

<210> 65
<211> 18
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<400> 65
gcttaacttc cgggttcg 18

<210> 66
<211> 18
<212> DNA
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<220>
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<400> 66
gcttaacttc taggttcg 18

<210> 67
<211> 18
<212> DNA
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<400> 67
gcttaacttc tgggttcg 18

<210> 68
<211> 18
<212> DNA
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<220>
<223> Description of the artifical sequence: consensus sequence

<400> 68
gcttaacttc caggttcg 18

<210> 69
<211> 18
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: consensus sequence

<400> 69
gcttaacttc cgagttcg 18

<210> 70
<211> 18
<212> DNA
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<220>
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<400> 70
gcttaacttc taagttcg 18

<210> 71
<211> 18
<212> DNA
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<220>
<223> Description of the artifical sequence: consensus sequence

<400> 71
gcttaacttc tgagttcg 18

<210> 72
<211> 18
<212> DNA
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<220>
<223> Description of the artifical sequence: consensus sequence

<400> 72
gcttaacttc caagttcg 18

<210> 73
<211> 25
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific

sequence for *Lactobacillus brevis*

<400> 73

tcgagaataa ttgaataata tctag

25

<210> 74

<211> 20

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Lactobacillus brevis*

<400> 74

gaggaagaa gttctcttat

20

<210> 75

<211> 23

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Lactobacillus lindneri*

<400> 75

aacagagaag atattatcta gtt

23

<210> 76

<211> 42

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Lactobacillus lindneri*

<400> 76

ttgagagaac gaagttcgct caggcttatg aaaaataagc at

42

<210> 77

<211> 45

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Lactobacillus casei*

<400> 77

ttcgttggcc gggttttggc caatggattc agggttctta tgtgg

45

<210> 78
<211> 58
<212> DNA
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<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus casei

<400> 78
gcgttttcgat gaaatacact ggttcccgac aacacaaaaa caacaatgat agccagtt 58

<210> 79
<211> 29
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus casei and Lactobacillus
paracasei

<400> 79
ttagaaaccg gagcataagc gggcctgag 29

<210> 80
<211> 46
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus paracasei

<400> 80
gcgtgatggc cgggctttgg ccattgcggt cagggtcctt atgtgc 46

<210> 81
<211> 46
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Lactobacillus paracasei

<400> 81
caagtacgtt aagttcaagg cagcaattaa acaatgatag ctagtt 46

<210> 82
<211> 44
<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for Lactobacillus coryniformis

<400> 82

aaagaaatga atatccagtt ttgagagcgc aacgttctca gaaa

44

<210> 83

<211> 48

<212> DNA

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<223> Description of the artifical sequence: specific
sequence for Lactobacillus curvatus

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aggtgcaatg ttaggctttt gaaatgaaat attacttatt atgcagtt

48

<210> 84

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<223> Description of the artifical sequence: specific
sequence for Pediococcus damnosus

<400> 84

gccgcgtaag tggatcggag aa

22

<210> 85

<211> 22

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for Pediococcus inopinatus

<400> 85

gccgcggaag tggatcggag aa

22

<210> 86

<211> 25

<212> DNA

<213> Artifical sequence

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<223> Description of the artifical sequence: sequence for
the detection of Pediococcus damnosus, Pediococcus

inopinatus and *Pediococcus parvulus*

<400> 86

gagagaataa atttctttca cacga

25

<210> 87

<211> 39

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus cerevisiiphilus*

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aaaatcatcg aaaaaaatgt ttggtctgag atttcttct

39

<210> 88

<211> 25

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus cerevisiiphilus*

<400> 88

cactctgggt gaagggcagg gaacg

25

<210> 89

<211> 39

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus frisingensis*

<400> 89

gatttcatca aaaaagagaa atgtttgggtc agagatttt

39

<210> 90

<211> 33

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for *Pectinatus frisingensis*

<400> 90

tatataccgg ctgaggtgct gaggcactga agg

33

<210> 91
<211> 36
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<223> Description of the artifical sequence: specific
sequence for Pectinatus spec. DSM 20764

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36

<210> 92
<211> 54
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Pectinatus spec. DSM 20764

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agattagttc ctggtttact ttatatatga gcactaaggt gcagaaaaga acgt

54

<210> 93
<211> 20
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Pectinatus spec. DSM 20764

<400> 93
aggaaacgcg gcgttcgtaa

20

<210> 94
<211> 56
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for Selenomonas lacticifex

<400> 94
taataatcta gaatgtttcg atacaatttt tcttctgtat agttttgagt ggacat

56

<210> 95
<211> 24
<212> DNA
<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for Zymophilus raffinovorans

<400> 95

gaggcgaaag cggaaggcag cgat

24

<210> 96

<211> 24

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for Zymophilus paucivorans

<400> 96

gaggcgaaag ctaaaggcag cgat

24

<210> 97

<211> 37

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: specific
sequence for Megasphaera cerevisiae

<400> 97

aatcctgaaa cgaattcagt ggtgatggct gcaggga

37

<210> 98

<211> 20

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: sequence for
detection of bacteria of the family Lactobacillaceae
that are relevant to brewing

<400> 98

tatggaagta agacccctga

20

<210> 99

<211> 21

<212> DNA

<213> Artifical sequence

<220>

<223> Description of the artifical sequence: sequence for

detection of bacteria of the family Lactobacillaceae
that are relevant to brewing

<400> 99

agatgatkag gtagataggc t

21

<210> 100

<211> 21

<212> DNA

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<223> Description of the artifical sequence: sequence for
detection of bacteria of the family Lactobacillaceae
that are relevant to brewing

<400> 100

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21

<210> 101

<211> 21

<212> DNA

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<223> Description of the artifical sequence: sequence for
detection of bacteria of the family Lactobacillaceae
that are relevant to brewing

<400> 101

agatgatkag gtagatagg t

21

<210> 102

<211> 25

<212> DNA

<213> Artificial sequence

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<223> Description of the artifical sequence: sequence for
detection of bacteria of the family Lactobacillaceae
that are relevant to brewing

<400> 102

tactaatcgg tcgaggactt aacca

25

<210> 103

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artifical sequence: sequence for
detection of bacteria of the family Lactobacillaceae

that are relevant to brewing

<400> 103
atactaata gtcgaggact taacca 26

<210> 104
<211> 32
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sequence for the genus Pectinatus

<400> 104
gaagcggact ggtactaata agccgagagc tt 32

<210> 105
<211> 32
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for the genus Selenomonas

<400> 105
cagcggacca atactaataa atcgagggct ta 32

<210> 106
<211> 38
<212> DNA
<213> Artifical sequence

<220>
<223> Description of the artifical sequence: specific
sequence for the genus Zymophilus

<400> 106
agcggaccga tactaatagg tcgaggggctt gacttaaa 38

<210> 107
<211> 32
<212> DNA
<213> Artifical sequence

<220>
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sequence for the genus Megasphaera

<400> 107
ggagcggacc ggtactaata gaccgaggac tt 32

SEQUENZPROTOKOLL

<110> BioteCon Diagnostics GmbH

<120> Verfahren und Nukleinsäuren zum Nachweis von
brauereirelevanten Mikroorganismen

<130> PCT1218-066

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<160> 107

<170> PatentIn Ver. 2.1

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<211> 326

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<213> Lactobacillus lindneri

<400> 2

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<210> 3

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<212> DNA

<213> Lactobacillus casei

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PCT/EP00/08808

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 <212> DNA
 <213> *Lactobacillus paracasei*

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 cgccgagagt agttgggtggg aaactgcctg cgaggata 338

<210> 6
 <211> 317
 <212> DNA
 <213> *Lactobacillus coryniformis* ssp. *coryniformis*

<400> 6
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 tgagagcgca acgttctcag aaagtgggtg ggtggcgata gcaagaagga tacacctgtt 240
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 tgcgaggata ggacgat 317

<210> 7
 <211> 317
 <212> DNA
 <213> *Lactobacillus coryniformis* ssp. *torquens*

<400> 7
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PCT/EP00/08808

<210> 8
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 <213> *Lactobacillus curvatus*

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 ttggtgggaa actacctgag aggataggac gatggt 336

<210> 9
 <211> 335
 <212> DNA
 <213> *Pediococcus damnosus*

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 accacaaagt ggtgttctca agagaaggat tcgatattat ttagtcttga gagaataaat 180
 ttctttcaca cgagccgagc aagtggatcg gagaagtgtg gtgacgatag tgagaaggat 240
 acacctgttc ccatgtcgaa cacagaagtt aagcttctta acgccgagag tagttggggg 300
 atcgctccct gcgaggatag gacgatggtc aatag 335

<210> 10
 <211> 326
 <212> DNA
 <213> *Pediococcus inopinatus*

<400> 10
 agatgagatt tcccattcca tttatggaag taagaccctt gagagatgat caggtagata 60
 ggttgggagtg ggaagtgtag tgatacatgg agcggaccaa tactaatcgg tcgaggactt 120
 aaccacaaag tgggtgttctc aaagagaaga tttcgatatt atttagtttt gagagaataa 180
 atttctttca cagagccgc ggaagtggat cggagaagtg tggtagcatg agtgagaagg 240
 atacacctgt tcccatgtcg aacacagaag ttaagcttct taacgccgag agtagttggg 300
 ggatcgctcc ctgagaggat aggacg 326

<210> 11
 <211> 403
 <212> DNA
 <213> *Pectinatus cerevisiiphilus*

<400> 11
 aagtgtgtaa agcatctaag cgtgaaacct gccttaagat gaggtttccc agagccgtaa 60
 ggcttggaag gcaccttgaa taagacgagg tagataggcc gggagtagaa gtacagtaat 120
 gtacgaagcg gactggtact aataagccga gagcttaact taaaatcatc gaaaaaatg 180
 tttggtctga gatttcttct gtgaagtttt gtagtgcaa gacactctgg ttgaagggca 240
 gggaacgtga gagcgtaaaa ctgaggactt tggctcaaag agttaagca tctggtgacg 300
 atacctggat ggatccacct gttcccatc cgaacacagt agttaagcat ccacaggctg 360
 aagggtacttg gggggcgacc ccctgggaaa ataggacact gcc 403

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<210> 12
 <211> 434
 <212> DNA
 <213> *Pectinatus frisingensis*

<400> 12
 aagtgctgaa agcatctaag cgtgaaacca gctttaagat gaggtttccc agaacgcaag 60
 tttggaaggc accttgaaga agacgaggta gataggccgg gagtggaagt atggtgacat 120
 atgaagcggg ctggtactaa taagccgaga gcttaacttg atttcatcaa aaaagagaaa 180
 tgtttggtca gagattttct tctgtgaagt tttgagtgtg caagaacact cgagagtata 240
 taggtaaagg aaaagcagca gataagtttc ctggttactg tatataccgg ctgaggtgct 300
 gaggcactga aggccagaac atctgggtggc gatacctgga tggatccacc tgttcccatt 360
 ccgaacacag tagttaagca tccacaggcc gaaggtaact ggggggcagc cccctgcgaa 420
 aataggacac cgcc 434

<210> 13
 <211> 641
 <212> DNA
 <213> *Pectinatus spec. DSM20764*

<400> 13
 aagtgctgaa agcatctaag cgtgaaacct gccttaagat gaggtttccc agagccgtaa 60
 ggcttggaag gcaccttgaa gatgacgagg tagataggcc gggagtagaa gtatggtgac 120
 atacgaagcg gactggtact aataagccga gagcttaact taatttcac tataaatggt 180
 tggtcctgat ttcttctgtg aagttttgag tgtgcaagat cactcatgaa agtatatagg 240
 taaagggaaa gcagcagatt agttcctggt ttactttata tatgagcact aagggtgcaga 300
 aaagaacggt tgaggaaacg cggcgttcgt aaactcactt tgcgtgctga ttatctcaat 360
 gctaaagcat taagataatt ttagaggaaa cgcgcgttca ctacggttca ctctgcgtac 420
 tttatttcta agtgctgaag cactaagaag ggcaaggaaa cgcgtcgttc gcgatgctca 480
 ctttgcgtag ttcatctcta gactgctaaa gcagtaagat ctgaagcatc tgggtggcgat 540
 acctggatgg atccacctgt tcccattccg aacacagtag ttaagcatcc acaggccgaa 600
 ggtacttggg gggcagcccc ctgcgagagt aggacatcgc c 641

<210> 14
 <211> 495
 <212> DNA
 <213> *Pectinatus spec. DSM20764*

<400> 14
 aagtgctgaa agcatctaag cgtgaaacct gccttaagat gaggtttccc agagccgtaa 60
 ggcttggaag gcaccttgaa gatgacgagg tagataggcc gggagtagaa gtatggtgac 120
 atacgaagcg gactggtact aataagccga gagcttaact taatttcac tataaatggt 180
 tggtcctgat ttcttctgtg aagttttgag tgtgcaagat cactcatgaa agtatatagg 240
 taaaggggaa gcagattagt tcctgggttta ctttatatat gagcactaag gtgcagaaaa 300
 gaacgtctaa ggaaacgcgg cgttcgtagg ctactctgc gtacttcac tctagactgc 360
 taaagcagta agatctgaag catctggtgg cgatacctgg atggatccac ctgttcccat 420
 tccgaacaca gtagttaagc atccacaggc cgaaggtaact tggggggcag cccctgcga 480
 aagtaggaca ccgcc 495

<210> 15
 <211> 546
 <212> DNA
 <213> *Megasphaera cerevisiae*

<400> 15

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gcatctaagc gtgaaaccag cctagagatg aggttttctca ttacgaaagt aagtaaggtc 60
ccatgaagac gacatggtag ataggccggg agtggacgta cagtaatgta tggagcggac 120
cggactaat agaccgagga cttgacttaa gcagggaacc catttttaaag aagcgaagcg 180
acgcataaaa tggagtgagt cgcttatacc gaatcgcaga ttcggtaaag cagcggagaa 240
taccaatgca gcggaacac cagttagcat aaactaagcg gattcggagt gggtgaggga 300
gtttcgtagc agcgtaggct aacccaacca ccgctttcga agaaggcgaa tggtttgaaa 360
aagagtacat gcaagaaac gacgaaagac tcacaaccaa aacatacaaa ctaagtagat 420
gacattagag tcacaccgat tgtaagatc cgaaatactt ttcgatgtag ttgtcaggat 480
acgaatcctg aaacgaattc agtgggtgatg gctgcaggga tccacctgtt cccataccga 540
acacag
546

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<210> 16

<211> 306

<212> DNA

<213> *Megasphaera cerevisiae*

<400> 16

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gcatctaacc gtgaaaccag cctagagatg aggttttctca ttacgaaagt aagtaaggtc 60
ccatgaagac gacatggtag ataggccggg agtggacgta cagtaatgta tggagcggac 120
cggactaat agaccgagga cttgacttaa gcaaagaagc aatagaaaga accatgtaga 180
tggtgtaaga gttagacggg tagttaaggc ccgaaatact tttcgatgta gttgtcagga 240
tacgaatcct gaaacgaatt cagtgggtgat ggctgcaggg accacctgtt cccataccga 300
acacag
306

```

<210> 17

<211> 449

<212> DNA

<213> *Selenomonas lacticifex*

<400> 17

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aagtgctgaa agcatctagg cgtgaagcct gtcccgagat gaagtatctc atggagtaat 60
ccagtaagat tccttgaaga agacaaggta gatagggttg gagtgtaagc atcgtaaggc 120
gttcagcggg ccaataactaa taaatcgagg gcttaacttt acagacctgt ccaagaagcg 180
aagcggattg ggtaacaggc cgtatgcgaa aacatcccaa gaatcgagtc cgaagggcga 240
agatgattgg cagatgttga ccgctaataa tctagaatgt ttcgatacaa tttttcttct 300
gtatagtttt gagtggacat cgttcattca ataatatcca gtgacgatag ctgagtggta 360
ccacctgttc ccataccgaa cacagtagtt aagcactcat acgccgaaag tacttgtctg 420
gaaacgggct gcgagaatag gacgtcgcc
449

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<210> 18

<211> 343

<212> DNA

<213> *Selenomonas lacticifex*

<400> 18

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aagtgctgaa agcatctaag cgtgaagcct gtcccgagat gaagtatctc atggagtaat 60
ccagtaagat tccttgaaga agacaaggta gatagggttg gagtgtaagc atcgtaaggc 120
gttcagcggg ccaataactaa taaatcgagg gcttatctta ataactaga atgtttcgat 180
acaatttttc ttctgtatag ttttgagtgg acatggttca ttcaataata tccagtgcgc 240
atagctgagt ggtaccacct gttcccatac cgaacacagt agttaagcac tcatacgccg 300
aaagtacttg tctggaaacg ggctgcgaaa ataggacgcc gcc
343

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<210> 19
 <211> 395
 <212> DNA
 <213> *Zymophilus raffinovorans*

<400> 19
 aagtgctgaa agcatctaag cgtgaaacca gccttaagat gaggtttctc acagagcaat 60
 ctggtaagac cccttgaaga agacaaggta gataggtcgg gagggaagc gcagtaatgt 120
 gtgcagcggg ccgatactaa taggtcgagg gcttgactta aagccagaac gaaaactaaa 180
 atgcgaacat ttctttcttc tgtatagttt tgagagaaca aactcttaag atggagtagt 240
 ctgaggcgaa agcgggaaggc agcgatatct aaaaaaagaa tatctggtag tgatagccaa 300
 gtggaccac ctgttcccat accgaacaca gtagttaagc acttgaacgt cgaaagtact 360
 tgggtggaaa cgccctgcga aaataggaca ccgcc 395

<210> 20
 <211> 395
 <212> DNA
 <213> *Zymophilus paucivorans*

<400> 20
 aagtgctgaa agcatctaag cgtgaaacca gccttaagat gaggtttctc acagagcaat 60
 ctggtaagac cccttgaaga agacaaggta gataggtcgg gagggaagc gcagtaatgt 120
 gtgtagcggg ccgatactaa taggtcgagg gcttgactta aagccagaac gaattctaaa 180
 atgcgaacat ttctttcttc tgtatagttt tgagagaaca gactcttaag atgagcagtc 240
 tgaggcgaaa gctaaaggca gcgatatcta aaaaaaagaa tatctggtag tgatagccaa 300
 gtggaccac ctgttcccat accgaacaca gtagttaagc acttgaacgt cgaaagtact 360
 tgggtggaaa cgccctggga aaataggaca ccgcc 395

<210> 21
 <211> 21
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus brevis*

<400> 21
 ccaagtcaac aacgtagttg t 21

<210> 22
 <211> 23
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus lindneri*

<400> 22
 gacacagggt taaatcaaag ttg 23

<210> 23

<211> 20
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Lactobacillus casei* und *Lactobacillus*
paracasei

<400> 23
aggtttctgc gactgcgaac

20

<210> 24
<211> 25
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Lactobacillus coryniformis*

<400> 24
atgtacgtag tgtagttta agggc

25

<210> 25
<211> 20
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Lactobacillus curvatus*

<400> 25
cttctcagtg cgcaagcaca

20

<210> 26
<211> 22
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pediococcus damnosus*

<400> 26
gtgttctcaa gagaaggatt cg

22

<210> 27
<211> 27
<212> DNA
<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pediococcus inopinatus*

<400> 27

gttctcaaag agaagatttc gatatta

27

<210> 28

<211> 23

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus cerevisiiphilus*

<400> 28

tgagagcgta aaactgcgga ctt

23

<210> 29

<211> 22

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus frisingensis*

<400> 29

cagataagtt tcctggttac tg

22

<210> 30

<211> 23

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus spec. DSM 20764*

<400> 30

cactaagggtg cagaaaagaa cgt

23

<210> 31

<211> 26

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Megasphaera cerevisiae*

<400> 31

cttttcgatg tagttgtcag gatacg

26

<210> 32

<211> 25

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Selenomonas lacticifex*

<400> 32

gttcattcaa taatatccag tgacg

25

<210> 33

<211> 23

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Zymophilus raffinosivorans*

<400> 33

aactcttaag atggagyagt ctg

23

<210> 34

<211> 22

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Zymophilus paucivorans*

<400> 34

actcttaaga tgagcagtct ga

22

<210> 35

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung *Pediococcus*

<400> 35

agtstagtga tacatggagc g

21

<210> 36
<211> 22
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Pectinatus

<400> 36
gtgaagtttt gagtgtgcaa ga

22

<210> 37
<211> 22
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Megasphaera

<400> 37
gaccgaggac ttgacttaag ca

22

<210> 38
<211> 20
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Selenomonas

<400> 38
tccagtgcgc atagctgagt

20

<210> 39
<211> 25
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Zymophilus

<400> 39
aagaatatct ggtagtgata gccaa

25

<210> 40
<211> 19
<212> DNA
<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 40

gtcgtgagac agttcggtc

19

<210> 41

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 41

cytagtacga gaggaccggr r

21

<210> 42

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 42

gctaccctgg ggataacagg c

21

<210> 43

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 43

atcgacgggg aggtttssca c

21

<210> 44

<211> 20

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 44
cacctcgatg tcggctcrtc

20

<210> 45
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 45
ccaagggttg ggctgttc

18

<210> 46
<211> 19
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 46
aagggccatc rctcaacgg

19

<210> 47
<211> 20
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 47
aagtgtgtaa agcatctaag

20

<210> 48
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 48
tgtgttcgii atgggaacag gtg

23

<210> 49
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 49
tgtgttcgga atgggaacag gtg

23

<210> 50
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 50
tgtgttcgaa atgggaacag gtg

23

<210> 51
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 51
tgtgttcggt atgggaacag gtg

23

<210> 52
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 52
tgtgttcgat atgggaacag gtg

23

<210> 53
<211> 23
<212> DNA
<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 53

tgtgttcggc atgggaacag gtg

23

<210> 54

<211> 23

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 54

tgtgttcgac atgggaacag gtg

23

<210> 55

<211> 19

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 55

ggcrrygtcc taytytcsc

19

<210> 56

<211> 19

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 56

ggcagtgtcc tactttccc

19

<210> 57

<211> 19

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 57

ggcagcgtcc tactttcgc

19

<210> 58

<211> 19

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 58

ggcagtgtcc tactttcgc

19

<210> 59

<211> 19

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 59

ggcagcgtcc tactttccc

19

<210> 60

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 60

gyttmrcttc yrdgttcg

18

<210> 61

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 61

gcttaacttc cgtgttcg

18

<210> 62
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 62
gcttaacttc tatgttcg

18

<210> 63
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 63
gcttaacttc tgtgttcg

18

<210> 64
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 64
gcttaacttc catgttcg

18

<210> 65
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 65
gcttaacttc cgggttcg

18

<210> 66
<211> 18
<212> DNA
<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 66

gcttaacttc taggttcg

18

<210> 67

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 67

gcttaacttc tgggttcg

18

<210> 68

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 68

gcttaacttc caggttcg

18

<210> 69

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 69

gcttaacttc cgagttcg

18

<210> 70

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 70

gcttaacttc taagtctg

18

<210> 71

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 71

gcttaacttc tgagtctg

18

<210> 72

<211> 18

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz:
Consensus-Sequenz

<400> 72

gcttaacttc caagtctg

18

<210> 73

<211> 25

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Lactobacillus brevis*

<400> 73

tgcgaataa ttgaataata tctag

25

<210> 74

<211> 20

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Lactobacillus brevis*

<400> 74

gaggaagaa gttctcttat

20

<210> 75
 <211> 23
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus lindneri*

<400> 75
 aacagagaag atattatcta gtt

23

<210> 76
 <211> 42
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus lindneri*

<400> 76
 ttgagagaac gaagttcgct caggcttatg aaaaataagc at

42

<210> 77
 <211> 45
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus casei*

<400> 77
 ttcgttggcc gggttttggc caatggattc agggttctta tgtgg

45

<210> 78
 <211> 58
 <212> DNA
 <213> Künstliche Sequenz

<220>
 <223> Beschreibung der künstlichen Sequenz: spezifische
 Sequenz für *Lactobacillus casei*

<400> 78
 gcgtttcgat gaaatacact gggtcccgac aacacaaaaa caacaatgat agccagtt

58

<210> 79
 <211> 29
 <212> DNA
 <213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische Sequenz für *Lactobacillus casei* und *Lactobacillus paracasei*

<400> 79

ttagaaaccg gagcataagc gggcctgag

29

<210> 80

<211> 46

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische Sequenz für *Lactobacillus paracasei*

<400> 80

gcgtgatggc cgggctttgg ccattgcggt cagggtcctt atgtgc

46

<210> 81

<211> 46

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische Sequenz für *Lactobacillus paracasei*

<400> 81

caagtacgtt aagttcaagg cagcaattaa acaatgatag ctagtt

46

<210> 82

<211> 44

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische Sequenz für *Lactobacillus coryniformis*

<400> 82

aaagaaatga atatccagtt ttgagagcgc aacgttctca gaaa

44

<210> 83

<211> 48

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische Sequenz für *Lactobacillus curvatus*

<400> 83

aggtgcaatg ttaggctttt gaaatgaaat attacttatt atgcagtt

48

<210> 84

<211> 22

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pediococcus damnosus*

<400> 84

gccgcgtaag tggatcggag aa

22

<210> 85

<211> 22

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pediococcus inopinatus*

<400> 85

gccgcggaag tggatcggag aa

22

<210> 86

<211> 25

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis von *Pediococcus damnosus*, *Pediococcus*
inopinatus und *Pediococcus parvulus*

<400> 86

gagagaataa atttctttca cacga

25

<210> 87

<211> 39

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus cerevisiiphilus*

<400> 87

aaaatcatcg aaaaaaatgt ttggtctgag atttcttct

39

<210> 88
<211> 25
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus cerevisiiphilus*

<400> 88
cactctgggtt gaagggcagg gaacg 25

<210> 89
<211> 39
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus frisingensis*

<400> 89
gatttcatca aaaaagagaa atgtttgggtc agagatttt 39

<210> 90
<211> 33
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus frisingensis*

<400> 90
tatataccgg ctgaggtgct gaggcactga agg 33

<210> 91
<211> 36
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus spec. DSM 20764*

<400> 91
aatttcatct ataaatgttt ggtcctgatt tcttct 36

<210> 92
<211> 54
<212> DNA
<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus spec.* DSM 20764

<400> 92

agattagttc ctggtttact ttatatatga gcactaaggt gcagaaaaga acgt 54

<210> 93

<211> 20

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Pectinatus spec.* DSM 20764

<400> 93

aggaaacgcg gcgttcgtaa 20

<210> 94

<211> 56

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Selenomonas lacticifex*

<400> 94

taataatcta gaatgtttcg atacaatttt tcttctgtat agttttgagt ggacat 56

<210> 95

<211> 24

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Zymophilus raffinosivorans*

<400> 95

gaggcgaaag cggaaggcag cgat 24

<210> 96

<211> 24

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Zymophilus paucivorans*

<400> 96

gaggcgaaag ctaaaggcag cgat

24

<210> 97

<211> 37

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für *Megasphaera cerevisiae*

<400> 97

aatcctgaaa cgaattcagt ggtgatggct gcaggga

37

<210> 98

<211> 20

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der *Lactobacillaceae*

<400> 98

tatggaagta agaccctga

20

<210> 99

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der *Lactobacillaceae*

<400> 99

agatgatcag gtagataggc t

21

<210> 100

<211> 21

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der *Lactobacillaceae*

<400> 100

agatgatcag gtcgataggt t

21

<210> 101
<211> 21
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der Lactobacillaceae

<400> 101
agatgatcag gtagataggt t

21

<210> 102
<211> 25
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der Lactobacillaceae

<400> 102
tactaatcgg tcgaggactt aacca

25

<210> 103
<211> 26
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: Sequenz zum
Nachweis brauereirelevanter Bakterien der Familie
der Lactobacillaceae

<400> 103
atactaatca gtcgaggact taacca

26

<210> 104
<211> 32
<212> DNA
<213> Künstliche Sequenz

<220>
<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Pectinatus

<400> 104
gaagcggact ggtactaata agccgagagc tt

32

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<210> 105

<211> 32

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Selenomonas

<400> 105

cagcggacca atactaataa atcgagggct ta

32

<210> 106

<211> 38

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Zymophilus

<400> 106

agcggaccga tactaatagg tcgagggctt gacttaaa

38

<210> 107

<211> 32

<212> DNA

<213> Künstliche Sequenz

<220>

<223> Beschreibung der künstlichen Sequenz: spezifische
Sequenz für die Gattung Megasphaera

<400> 107

ggagcggacc ggtactaata gaccgaggac tt

32

Patent Claims

1. Method for the detection of microorganisms relevant to brewing in a sample, which comprises the following steps:

- (a) bringing the sample into contact with a combination of at least two first nucleic acid molecules (primers), which hybridise with a region of a microbial nucleic acid conserved in microorganisms relevant to brewing;
- (b) amplification of the microbial nucleic acid or a portion thereof to produce at least one amplification fragment;
- (c) bringing the amplification fragments obtained in step (b) into contact with at least one second nucleic acid molecule (probe), which specifically hybridises with at least one amplification fragment that comprises a sequence of the microbial nucleic acid specific for all microorganisms relevant to brewing or for one or several families, genera or species of microorganisms relevant to brewing;
- (d) detection of at least one hybrid nucleic acid which consists of an amplification fragment and a second nucleic acid molecule introduced in step (c).

2. Method according to Claim 1, characterised in that the amplification comprises a polymerase chain reaction (PCR).

3. Method according to Claim 1, characterised in that the amplification comprises a ligase chain reaction.

4. Method according to Claim 1, characterised in that the amplification comprises an isothermal nucleic acid amplification.

5. Method according to one of Claims 1 to 4, characterised in that the second nucleic acid molecule is modified or labelled to produce a detectable signal, the modification or labelling being selected from (i) radioactive groups, (ii) coloured groups, (iii) fluorescent groups, (iv) groups for immobilisation on a solid phase and (v) groups which allow an

indirect or direct reaction, particularly by means of antibodies, antigens, enzymes and/or substances with affinity for enzymes or enzyme complexes.

6. Method according to one of the preceding Claims, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule are at least 10 nucleotides, preferably 15-30 nucleotides long.

7. Method according to one of the preceding Claims, characterised in that the first nucleic acid molecule and/or the second nucleic acid molecule is modified in that up to 20% of the nucleotides in 10 consecutive nucleotides, in particular 1 or 2 nucleotides from the block of 10 are replaced by nucleotides which do not naturally occur in bacteria.

8. Method according to one of the preceding Claims, characterised in that the conserved region occurs in the genome section which contains the bacterial 23 S and 5 S genes.

9. Nucleic acid molecule as probe and/or primer for the detection of microorganisms relevant to brewing, said nucleic acid molecule being selected from:

- (i) a nucleic acid with a sequence according to SEQ ID NO 1-107 or a fragment thereof at least 10, preferably 15-30 nucleotides long;
- (ii) a nucleic acid which specifically hybridises with a nucleic acid according to (i);
- (iii) a nucleic acid which is at least 70%, preferably at least 90%, identical with a nucleic acid according to (i) or (ii), or
- (iv) a nucleic acid which is complementary to a nucleic acid according to (i) to (iii).

10. Nucleic acid molecule according to Claim 9, characterised in that it is a DNA or an RNA.

11. Nucleic acid molecule according to Claim 9, characterised in that it is a PNA.

12. Nucleic acid molecule according to Claim 9 to 11, characterised in that up to 20% of the nucleotides in 10 consecutive nucleotides, in particular 1 or 2 nucleotides from the block of 10 are replaced by nucleotides which do not occur naturally in bacteria.

13. Combination of at least two nucleic acid molecules, said combination being selected from:

- (1) a combination of at least two nucleic acid molecules according to one of Claims 9 to 12, and
- (2) a combination which comprises at least one nucleic acid molecule with a sequence according to one of the SEQ ID NO 40 to 47 and at least one nucleic acid molecule with a sequence according to SEQ ID NO 48-54 or SEQ ID NO 55-59 or SEQ ID NO 60-72.

14. Kit containing a nucleic acid molecule according to one of Claims 9 to 12 and/or a combination according to Claim 13.

15. Method according to one of Claims 1 to 8, characterised in that in step (a) a combination of at least two nucleic acid molecules according to Claim 13 is used.

16. Method according to one of Claims 1 to 8 and 15, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule according to one of Claims 9 to 12 is used.

17. Method according to Claim 16, characterised in that as second nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NO 35 to 39 or 98-107 is used.

18. Method according to Claim 16 or 17, characterised in that as second or further nucleic acid molecule (probe) at least one nucleic acid molecule with a sequence according to one of SEQ ID NO 21 to 34 or SEQ ID NO 73-97 is used.

19. Use of a nucleic acid molecule according to one of Claims 9 to 12 and/or a combination according to Claim 13 for the detection of bacteria relevant to brewing.

20. Use of a nucleic acid molecule according to one of Claims 9 to 12 for the identification and/or characterisation of bacteria relevant to brewing.

21. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 35 or SEQ ID NO 86 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Pediococcus*.
22. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 36 or SEQ ID NO 104 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Pectinatus*.
23. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 37 or SEQ ID NO 107 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Megasphaera*.
24. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 38 or SEQ ID NO 105 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Selenomonas*.
25. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 39 or SEQ ID NO 106 for the detection and/or for the identification and/or characterisation of bacteria of the genus *Zymophilus*.
26. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 1, SEQ ID NO 21 or SEQ ID NO 73-74 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus brevis*.
27. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 2, SEQ ID NO 22 or SEQ ID NO 75-76 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus lindneri*.
28. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 3, SEQ ID NO 23 or SEQ ID NO 77-79 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus casei*.

29. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 23 or SEQ ID NO 79-81 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus paracasei* ssp. *paracasei*.
30. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 6, SEQ ID NO 24 or SEQ ID NO 82 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus coryniformis* ssp. *coryniformis*.
31. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 7, SEQ ID NO 24 or SEQ ID NO 82 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus coryniformis* ssp. *torquens*.
32. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 8, SEQ ID NO 25 or SEQ ID NO 83 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Lactobacillus curvatus*.
33. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 9, SEQ ID NO 26, SEQ ID NO 84 or SEQ ID NO 86 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pediococcus damnosus*.
34. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 10, SEQ ID NO 27, SEQ ID NO 85 or SEQ ID NO 86 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pediococcus inopinatus*.
35. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 11, SEQ ID NO 28 or SEQ ID NO 87-88 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pectinatus cerevisiiphilus*.

36. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 12, SEQ ID NO 29 or SEQ ID NO 89-90 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Pectinatus frisingiensis*.
37. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 30 or SEQ ID NO 91-93 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the strain *Pectinatus* sp. DSM20764.
38. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 31 or SEQ ID NO 97 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Megasphaera cerevisiae*.
39. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 32 or SEQ ID NO 94 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Selenomonas lacticifex*.
40. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 19, SEQ ID NO 33 or SEQ ID NO 95 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Zymophilus raffinovorans*.
41. Use of a nucleic acid molecule with a sequence according to SEQ ID NO 20, SEQ ID NO 34 or SEQ ID NO 96 or a fragment thereof with at least 10, preferably 15-30 nucleotides for the detection and/or for the identification and/or characterisation of bacteria of the species *Zymophilus paucivorans*.