COMPOSITIONS FOR DETECTING FOODSTUFF SPOILAGE MICROORGANISMS

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 8,781 Byte ASCII (Text) file named "710675SequenceListing," dated July 20, 2012.

BACKGROUND OF THE INVENTION

[0002] Microorganisms (e.g., bacteria) may cause the spoilage of foodstuffs (e.g., beer) during or after manufacture. Some microorganisms may cause foodstuff spoilage and any one or more of several undesireable effects such as, for example, unpleasant odor, unpleasant taste, and rendering the foodstuff unsafe for consumption. Failure to accurately and rapidly detect the presence of foodstuff-spoiling microorganisms may increase the risk of food spoilage. Obstacles to the rapid and accurate detection of the microorganisms that cause the spoilage of foodstuffs may include, for example, the lengthy duration of the traditional microbiology methods used to detect the microorganisms. These traditional methods may take an average of 7-14 days to complete. Another obstacle to accurate detection may include, for example, the similarity of the genomic sequences of some foodstuff-spoiling microorganisms as compared to that of non-foodstuff-spoiling microorganisms.

[0003] Accordingly, there is a need for improved compositions and methods for detecting microorganisms that cause the spoilage of foodstuffs.

BRIEF SUMMARY OF THE INVENTION

[0004] An embodiment of the invention provides a nucleic acid consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45.

[0005] Another embodiment of the invention provides a collection of nucleic acids comprising two or more nucleic acids, wherein the two or more nucleic acids each consist of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45.

[0006] Another embodiment of the invention provides an assay kit comprising a collection of nucleic acids, wherein the collection is selected from the group consisting of (a) SEQ ID NOs: 1-3; (b) SEQ ID NOs: 4-6; (c) SEQ ID NOs: 7-9; (d) SEQ ID NOs: 10-12; (e) SEQ ID NOs: 13-15; (f) SEQ ID NOs: 16-18; (g) SEQ ID NOs: 19-21; (h) SEQ ID NOs: 22, 24, and 27; (i) SEQ ID NOs: 22, 25, and 27; (j) SEQ ID NOs: 23, 26, and 27; (k) SEQ ID NOs: 28-30; (l) SEQ ID NOs: 31-33; (m) SEQ ID NOs: 34-36; (n) SEQ ID NOs: 37-39; (o) SEQ ID NOs: 40-42; and (p) SEQ ID NOs: 43-45.

[0007] Still another embodiment of the invention provides a method of detecting the presence of one or more microorganisms in a foodstuff, the method comprising: (a) obtaining at least one test sample comprising isolated microorganism nucleic acid from foodstuff; (b) contacting the inventive nucleic acid, collection of nucleic acids, or support including at least one inventive nucleic acid with the at least one test sample under conditions allowing for a complex to form between the inventive nucleic acid and the microorganism nucleic acid; (c) detecting the complex; and, optionally, (d) comparing an amount of complex in the at least one test sample with an amount of complex from a negative sample that lacks microorganism nucleic acid, wherein an increased amount of complex from the at least one test sample is indicative of the presence of one or more microorganisms.

DETAILED DESCRIPTION OF THE INVENTION

[0008] An embodiment of the invention provides a nucleic acid consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45. In an embodiment, the nucleic acid is isolated or purified. The inventive nucleic acids provide forward primers, reverse primers, and probes which may, advantageously, specifically hybridize with a microorganism nucleic acid for detection of the presence of one or more microorganisms in a foodstuff. In some embodiments, the primers and probes specifically hybridize with the nucleic acid of a specific genus of microorganisms. In an especially preferred embodiment, the primers and probes specifically hybridize with the nucleic acid of a specific species of microorganisms.

[0009] The inventive nucleic acids may be used to detect the presence of one or more microorganisms that cause foodstuff spoilage in a sample of foodstuff. In an embodiment of the invention, the inventive nucleic acids may specifically detect one or more microorganisms of one or more genera selected from the group consisting of *Pediococcus,*

Lactobacillus, Pectinatus, and *Megasphaera.* Exemplary species of microorganisms specifically detectable by the inventive nucleic acids are set forth in Table 1.

TABLE 1

Assay Kit No.	**Species**	**Component**	**Sequence**	**SEQ ID NOs:**
1.	*Lactobacillus collinoides L. paracollinoides*	forward primer	ACGAACGCATCCCGTTAAA	1
		reverse primer	GGACCAGTTCGCCACTCATCC	2
		probe	CAAGTGCTTGCACGGATTTTAACATTG	3
2.	Inhibition Control	forward primer	CTTGCAAATCGTTCTTTGGG	4
		reverse primer	TAGCGGTACGACTGTCTTGG	5
		probe	TCAAACCCTAACCTCAGCTCCAGC	6
3.	*L. backii*	forward primer	TCCAAGTCGAACGCACAGATA	7
		reverse primer	GGGAAATGTTATCCCCCACTTTT	8
		probe	AGTGGCGGACGGGTGAGTAACACG	9
4.	*L. brevis*	forward primer	AACGAGCTTCCGTTGAATGAC	10
		reverse primer	CGGCCTGCTTCTGGGCAGA	11
		probe	TGCTTGCACTGATTTCAACAATGAAGC	12
5.	*Pectinatus (P. cerevisiiphilus, P. frisingensis, P. haikarae & P. portalensis)*	forward primer	TCAAGTTCTGTTGCAGGGGA	13
		reverse primer	ACCGGACAACGCTTGCCG	14
		probe	TGAATGACGGTACCCTGTTAGAAAGCC	15
6.	*L. lindneri*	forward primer	CGTCGAACGAGGTCTCCTAAC	16
		reverse primer	GCCTTCCAGGTGTTATCCCCTT	17
		probe	AGTGGCGAACTGGTGAGTAACACGT	18
7.	*Megasphaera (M. cerevisiae, M. elsdenii)*	forward primer	CGCGTGACGGTACCGTAAG	19
		reverse primer	GGAGCCCCGCACTTTTAAGAC	20
		probe	TACCGTAAGAGAAAGCCACGGCTAA	21
8.	*Pediococcus (P. damnosus, P. inopinatus, P. claussenii)*	forward primer *damnosus* and *inopinatus*	CGTAGAGATGCTTGCATCGAA	22
		forward primer *claussenii*	GGCTTGCACGGATAGATGATT	23

Assay Kit No.	Species	Component	Sequence	SEQ ID NOs:
		reverse primer *damnosus*	CCAACCATGCGGTTCATTTTA	24
		reverse primer *inopinatus*	CCAAACCATGCGGTTTACTTTA	25
		reverse primer *claussenii*	CCAACCATGCGGTTTTCTTTA	26
		probe	AGTGGCGAACGGGTGAGTAACACG	27
9.	*L. casei*	forward primer	TGGCTTGCACTGAGATTCGA	28
		reverse primer	CCACCATGCGGTTCTTGGAT	29
		probe	AGTGGCGGACGGGTGAGTAACACG	30
10.	*L. coryniformis*	forward primer	CAACGCACTGACGTCGACC	31
		reverse primer	AGCCAAAGGCCGTCTTTTACATT	32
		probe	AGTGGCGGACGGGTGAGTAACACG	33
11.	*L. parabuchneri (L. frigidus)*	forward primer	CCTGTTGAGTGCTTGCATTTAACTG	34
		reverse primer	GGCCAAGTGTTATCCCCTACTTCAA	35
		probe	AGTGGCGAACTGGTGAGTAACACGT	36
12.	*L. perolens*	forward primer	CCAGGTGCTTGCATCACCA	37
		reverse primer	TTTCCAAATGTTATCCCCTGCTG	38
		probe	AGTGGCGGACGGGTGAGTAACACG	39
13.	*L. plantarum*	forward primer	TGAAGTCGAACGAACTCTGGTA	40
		reverse primer	GCCCGAAGCCATCTTTCAAACTC	41
		probe	AGTGGCGAACTGGTGAGTAACACGT	42
14.	*L. rossiae*	forward primer	ACACGGTGCTTGCACCAGA	43
		reverse primer	GGCAAATGTTATCCCCCACTTTA	44
		probe	AGTGGCGAACGGGTGAGTAACACG	45

[0010] The inventive nucleic acids can specifically detect any type of microorganism nucleic acid. In an embodiment of the invention, the microorganism nucleic acid is DNA. In still another embodiment, the inventive nucleic acid is a nucleic acid consisting of a nucleotide sequence that is complementary to any of SEQ ID NOs: 1-45. The nucleic acid that is complementary to any of SEQ ID NOs: 1-45 may detect microorganism RNA.

[0011] In an embodiment, the inventive nucleic acid further comprises a detectable label. The label may be any label suitable for detecting hybridization, e.g., a complex, of the inventive nucleic acid with microorganism nucleic acid. Exemplary detectable labels may include any one or more of radioactive labels, non-radioactive labels, fluorescent labels, and chemiluminescent labels.

[0012] Another embodiment of the invention provides a collection of nucleic acids comprising two or more nucleic acids, wherein the two or more nucleic acids each consist of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45. In an embodiment of the invention, the collection may comprise or further comprise a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45. The collection may comprise any suitable number of inventive nucleic acids. For example, the collection may comprise from about 2 to about 45 or more nucleic acids, from about 10 or less to about 40 or more nucleic acids, or from about 20 or less to about 30 or more nucleic acids. In this regard, the collection may comprise 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, or 45 or more nucleic acids. Although the two or more nucleic acids of the collection may be identical to one another, in a preferred embodiment, the two or more nucleic acids are different from each other. Accordingly, the two or more different nucleic acids may, advantageously, hybridize with two or more different microorganism nucleic acids and, therefore, detect the presence of two or more different microorganisms in a foodstuff.

[0013] An embodiment of the invention provides an assay kit comprising a collection of nucleic acids for the specific detection of one or more species of one or more microorganisms. The collection of nucleic acids of the assay kit may include at least one primer and a probe, preferably at least one forward primer, at least one reverse primer, and at least one probe. In an embodiment, the assay kit is selected from the group consisting of assay kit numbers 1-14 of Table 1. Preferably, the collection of nucleic acids of the assay kit is selected from the group consisting of (a) SEQ ID NOs: 1-3; (b) SEQ ID NOs: 4-6; (c) SEQ ID NOs: 7-9; (d) SEQ ID NOs: 10-12; (e) SEQ ID NOs: 13-15; (f) SEQ ID NOs: 16-18; (g)

SEQ ID NOs: 19-21; (h) SEQ ID NOs: 22, 24, and 27; (i) SEQ ID NOs: 22, 25, and 27; (j) SEQ ID NOs: 23, 26, and 27; (k) SEQ ID NOs: 28-30; (l) SEQ ID NOs: 31-33; (m) SEQ ID NOs: 34-36; (n) SEQ ID NOs: 37-39; (o) SEQ ID NOs: 40-42; and (p) SEQ ID NOs: 43-45. In an embodiment, the assay kit may include sequences that are complementary to any one or more of SEQ ID NOs: 1-45.

[0014] By “nucleotide sequence” or "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the inventive nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0015] The inventive nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-

N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0016] An embodiment of the invention provides a support comprising the inventive nucleic acid, collection of nucleic acids, or collection of nucleic acids of the assay kit immobilized on the support. Another embodiment of the invention provides a support comprising the sample to be tested immobilized on the support, and the inventive nucleic acid is applied to the support. The support may be any support suitable for immobilizing the inventive nucleic acids. Exemplary supports are described in U.S. Patent Application No. 6,821,771, which is incorporated herein by reference in its entirety. Other exemplary supports include GENEDISC plates available from Pall Corporation, Port Washington, NY, USA.

[0017] The support may further comprise a detectable label. The label may be any label suitable for detecting a complex of the inventive nucleic acid with microorganism nucleic acid. Exemplary detectable labels may include any one or more of radioactive labels, non-radioactive labels, fluorescent labels, and chemiluminescent labels.

[0018] Still another embodiment of the invention provides a method of detecting the presence of one or more microorganisms in a foodstuff, the method comprising: (a) obtaining at least one test sample comprising isolated microorganism nucleic acid from foodstuff; (b) contacting the inventive nucleic acid, collection of nucleic acids, collection of nucleic acids of the assay kit, or support including at least one nucleic acid with the at least one test sample under conditions allowing for a complex to form between the inventive nucleic acid and the microorganism nucleic acid; (c) detecting the complex; and (d) comparing an amount of complex in the at least one test sample with an amount of complex from a negative sample that lacks microorganism nucleic acid, wherein an increased amount of complex from the at least one test sample is indicative of the presence of one or more microorganisms.

[0019] The method may comprise obtaining a sample of the foodstuff to be tested and culturing microorganisms in the sample in any suitable manner. For example, when the foodstuff is beer, the sample may be any one or more of clear beer, non-filtered beer, processed beer, fermented beer, or other turbid beer.

[0020] The method may comprise culturing the microorganisms in any suitable culturing medium as is known in the art. The culturing medium may be selected depending on the nature of the foodstuff and microorganism to be tested. Exemplary culturing media may include enrichment broth such as, for example, MRS enrichment broth available from AES CHEMUNEX, Inc., Cranbury, NJ, USA; and NBB-C enrichment broth available from VWR International, West Chester, PA, USA.

[0021] The microorganisms may be cultured at any suitable temperature and for any suitable duration as is known in the art. The culturing temperature and duration may be selected depending on the nature of the foodstuff and microorganism to be tested. For example, the microorganisms may be cultured at a temperature of about 20º C to about 40º C, preferably from about 22º C to about 28º C. The microorganisms may be cultured for about 1 day to about 14 days, preferably from about 2 days to about 7 days.

[0022] The method may comprise extracting nucleic acid from the microorganisms in any suitable manner as is known in the art. The nucleic acid may be RNA and/or DNA. The protocol for extracting nucleic acid may be selected depending on the nature of the foodstuff, microorganism, and nucleic acid to be tested as is known in the art. Preferably, the nucleic acid is extracted in any manner that lyses Gram positive and Gram negative bacteria and which recovers a testable amount of DNA without using polymerase chain reaction (PCR) inhibitors. The nucleic acid extraction may be carried out using any of a variety of commercially available nucleic acid extraction kits according to the manufacturer's instructions. Exemplary DNA extraction kits may include, for example, PEFOOD kit (available from Pall Corporation, Port Washington, NY, USA).

[0023] The method comprises contacting the inventive nucleic acid, collection of nucleic acids, collection of nucleic acids of the assay kit, or support with the at least one test sample under conditions allowing for a complex to form between the inventive nucleic acid and the microorganism nucleic acid. In this regard, the method comprises contacting the sample of extracted nucleic acid with the inventive nucleic acid under conditions which allow the inventive nucleic acid to specifically hybridize with microorganism nucleic acid as is known in the art. The method may comprise amplifying the inventive nucleic acid and the microorganism nucleic acid using any suitable type of PCR as is known in the art.

[0024] The method comprises detecting the complex. The complex may be detected using, for example, a radioactive label or a dye as is known in the art. In a preferred embodiment, the method comprises measuring light emitted from a fluorescent dye using,

e.g., a laser. Detecting the complex may further comprise measuring the amount of complex formed.

[0025] In an embodiment, the method optionally comprises comparing an amount of complex in the at least one test sample with an amount of complex from a negative sample that lacks microorganism nucleic acid, wherein an increased amount of complex from the at least one test sample is indicative of the presence of one or more microorganisms. In this regard, the sample is negative for the foodstuff-spoiling microorganism if the amount of complex detected in the sample is no more than the amount of complex that is detected in a negative sample that is known to lack the microorganism nucleic acid. The sample is positive for the foodstuff-spoiling microorganism if the amount of complex detected in the sample is more than the amount of complex that is detected in a negative sample that is known to lack the microorganism nucleic acid.

[0026] The method may, advantageously, comprise testing for the presence of microorganisms in more than one different foodstuff sample simultaneously. In this regard, the at least one test sample may be two or more different samples tested sequentially or simultaneously, i.e., 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more samples sequentially or simultaneously.

[0027] The method may comprise detecting the presence of any one or more microorganisms that causes spoilage of foodstuff. In an embodiment of the invention, the method comprises detecting the presence of one or more microorganisms of one or more genera selected from the group consisting of *Pediococcus, Lactobacillus, Pectinatus,* and *Megasphaera.* In a preferred embodiment, the method comprises detecting the presence of one or more species of microorganisms selected from the group consisting of *Pediococcus damnosus, Pediococcus inopinatus, Pediococcus claussenii, Lactobacillus backii, Lactobacillus brevis, Lactobacillus casei, Lactobacillus coryniformis, Lactobacillus collinoides, Lactobacillus lindneri, Lactobacillus rossiae, Lactobacillus parabuchneri, Lactobacillus paracollinoides, Lactobacillus perolens, Lactobacillus plantarum, Pectinatus cerevisiiphillus, Pectinatus frisingensis, Pectinatus haikarae, Pectinatus portalensis, Megasphaera cerevisiae,* and *Megasphaera elsdenii.*

[0028] The method may comprise detecting microorganisms in any foodstuff. The foodstuff may be, for example, any one or more of dairy products; fats, oils, and fat emulsions; edible ices (including, e.g., sherbet and sorbet); fruits and vegetables (including,

e.g., mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera); seaweeds; nuts and seeds; confectioneries; cereals and cereal products; baked goods (e.g., bread); meat and meat products (including, e.g., poultry and game); fish and fish products (including mollusks, crustaceans, and echinoderms); eggs and egg products; sweeteners, including, e.g., honey; salts, spices, soups, sauces, salads, protein products; foodstuffs intended for particular nutritional uses; and beverages (e.g., beer and wine). In a preferred embodiment, the foodstuff is beer.

[0029] The term "isolated" as used herein means having been removed from its natural environment. The term "purified" as used herein means having been increased in purity, wherein "purity" is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, can be greater than 60%, 70% or 80%, 90% or can be 100%.

[0030] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0031] This example demonstrates the quantitative detection of *L. casei* using the inventive nucleic acids.

[0032] Genomic DNA is isolated from *L. casei* (American Type Culture Collection (ATCC) strain reference E-011808) using a DNA Extraction Kit (available from Pall Corporation, Port Washington, NY, USA). Bacterial DNA is diluted with 10mM Tris-pH 8.3 to a concentration of 83 pg/µL. Tris-pH 8.3 buffer (10 mM) is used as a no target control (NTC).

[0033] An *L. casei* assay kit (including a forward primer (SEQ ID NO: 28), a reverse primer (SEQ ID NO: 29), and a probe (SEQ ID NO: 30)) is used to test DNA from *L. casei* at 0 (NTC), 10, 1,000 or 100,000 copies per PCR well. The probe is labeled with 6-FAM fluorescent dye at 5' and BHQ1 quencher at 3' or ROX fluorescent dye at 5' and BHQ2 quencher at 3'.

[0034] A GENEDISC plate (available from Pall Corporation, Port Washington, NY, USA) is prepared according the manufacturer's instructions.

[0035] A quantitative (q) PCR mix kit ("Master Mix") (included in the GENEDISC detection kit, available from Pall Corporation, Port Washington, NY, USA) is prepared including SEQ ID NOs: 28-30. The barcode located on the GENEDISC plate and the

barcode on the identification card contained in the Master Mix bag are scanned using the barcode reader fitted to the GENEDISC cycler (available from Pall Corporation, Port Washington, NY, USA). The sample names are entered according to the manufacturer's instructions for the GENEDISC cycler. Six 1.5 mL microtubes corresponding to each of the GENEDISC plate sectors are labeled. The Master Mix is vortexed for 2 seconds, then briefly centrifuged for 2 seconds. Master Mix (37 µL) is added to each microtube. The microtubes are closed. The DNA samples are centrifuged in a bench centrifuge for 15 seconds.

[0036] A DNA sample (37 µL) is transferred to the corresponding microtube containing the Master Mix using a pipette. The tube is closed to prevent cross-contamination. The tubes are gently mixed for 2 seconds and then centrifuged for 2 seconds using a mini centrifuge. These steps are repeated for each of the other 5 samples. 72 µL from each microtube is added to the appropriate GENEDISC plate sector.

[0037] The GENEDISC plates are loaded. The filling cap is placed on the top of the GENEDISC plate, the cap is gently pressed to ensure that there is no leakage and the vacuum is started. When the GENEDISC cycler indicates that the vacuuming is 90% complete, the GENEDISC plate is tapped to remove any residual bubbles. The cap is removed after the vacuum is released. Mineral oil (4 drops) are loaded into each GENEDISC plate sector. The filling cap is placed on the GENEDISC plate and the vacuum is started. The cap is removed at the end of the vacuum cycle and is cleaned by wiping with 70% ethanol. The wells are examined to ensure that there are no partially or unevenly filled wells present that may cause the assay kit to be aborted.

[0038] The filling cap is replaced in the designated location. The GENEDISC plate is carefully inserted into the GENEDISC cycler and the lid of the GENEDISC cycler is closed. The PCR is run using the thermal cycling condition. The thermal cycling condition includes four temperatures 113 ºC, 107 ºC, 57 ºC, and 63°C. The cycling time is 70 seconds per cycle for 45 cycles. At the end of the PCR, the GENEDISC place is removed and discarded.

[0039] The data are analyzed. The *L. casei* assay (SEQ ID NOs: 28-30) quantitatively detects *L. casei* target DNA at 10, 1,000 and 100,000 copies per PCR well. There is no signal in the absence of target or with the "no template" control (NTC). NTC measures non-specific signal in the absence of target molecules. The inhibition control provides a positive PCR signal and measures the degree of PCR inhibition in the presence of sample or contaminant(s) in the sample.

EXAMPLE 2

[0040] This example demonstrates the specificity of the inventive nucleic acids.

[0041] Genomic DNA is isolated from 13 beer spoiling bacteria (Table 2) and diluted as described in Example 1. Tris-pH 8.3 buffer (10 mM) is used as a no target control (NTC). All bacterial strains are obtained from the American Type Culture Collection (ATCC).

TABLE 2

Strain reference	Bacteria	DNA batch
E-64028	*L. brevis*	0011111LBRE-DH
E-79105	*Pectinatus cerevisiiphilus*	0010212LPEC-DH
E-052900	*L. backii*	0011111LBAC-DH
E-991161	*L. lindneri*	0011111LLIN-DH
E-91459T	*Pediococcus damnosus*	0011111PEDD-DH
E-79111T	*Megasphaera cerevisiae*	0011011MEGC-DH
E-95503T	*L. frigidus*	0011111LFRI-DH
DSM 15814	*L. rossiae*	0011111LRO-DH
E-011808	*L. casei*	0011111LCAS-DH
E-991162	*L. collinoides*	0011111LCOL-DH
E-991163	*L. coryniformis*	0011111LCOR-DH
E-89345	*L. perolens*	0011111LPER-DH
E-71034	*L. plantarum*	0011111LPLA-DH

[0042] An *L. casei* assay kit (including a forward primer (SEQ ID NO: 28), a reverse primer (SEQ ID NO: 29), and a probe (SEQ ID NO: 30)) is used to test samples of DNA from each the 13 different bacteria of Table 2 according to the procedures of Example 1. All probes are labeled with 6-FAM fluorescent dye at 5' and BHQ1 quencher at 3' or ROX fluorescent dye at 5' and BHQ2 quencher at 3'.

[0043] The *L. casei* assay (SEQ ID NOs: 28-30) quantitatively detects *L. casei* target DNA at 10, 1,000 and 100,000 copies per PCR well. However, there is no positive signal in the presence of 100,000 copies of each of the other 12 bacteria of Table 2. Accordingly, the *L. casei* assay (SEQ ID NOs: 28-30) is highly specific for *L. casei*.

[0044] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were

individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0045] The use of the terms “a” and “an” and “the” and “at least one” and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0046] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A nucleic acid consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45.

2. A collection of nucleic acids comprising two or more nucleic acids, wherein the two or more nucleic acids each consist of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-45.

3. The collection according to claim 2, wherein the two or more nucleic acids include two or more different nucleic acids.

4. An assay kit comprising a collection of nucleic acids, wherein the collection is selected from the group consisting of

(a) SEQ ID NOs: 1-3;
(b) SEQ ID NOs: 4-6;
(c) SEQ ID NOs: 7-9;
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(j) SEQ ID NOs: 23, 26, and 27;
(k) SEQ ID NOs: 28-30;
(l) SEQ ID NOs: 31-33;
(m) SEQ ID NOs: 34-36;
(n) SEQ ID NOs: 37-39;
(o) SEQ ID NOs: 40-42; and
(p) SEQ ID NOs: 43-45.

5. A support comprising the nucleic acid according to claim 1, the collection of nucleic acids according to any one of claims 2-3, or the collection of nucleic acids of the assay kit of claim 4 immobilized on the support.

6. The support according to claim 5, further comprising a detectable label.

7. A method of detecting the presence of one or more microorganisms in a foodstuff, the method comprising:

(a) obtaining at least one test sample comprising isolated microorganism nucleic acid from foodstuff;

(b) contacting the nucleic acid according to claim 1, the collection of nucleic acids according to any one of claims 2-3, the collection of nucleic acids of the assay kit according to claim 4, or the support including the nucleic acid according to claim 5 or 6 with the at least one test sample under conditions allowing for a complex to form between the nucleic acid and the microorganism nucleic acid;

(c) detecting the complex; and, optionally,

(d) comparing an amount of complex in the at least one test sample with an amount of complex from a negative sample that lacks microorganism nucleic acid, wherein an increased amount of complex from the at least one test sample is indicative of the presence of one or more microorganisms.

8. The method according to claim 7, comprising detecting the presence of one or more microorganisms that causes spoilage of foodstuff.

9. The method according to claim 7 or 8, comprising detecting the presence of one or more microorganisms of one or more genera selected from the group consisting of *Pediococcus, Lactobacillus, Pectinatus,* and *Megasphaera.*

10. The method according to any one of claims 7-9, comprising detecting the presence of one or more microorganisms selected from the group consisting of *Pediococcus damnosus, Pediococcus inopinatus, Pediococcus claussenii, Lactobacillus backii, Lactobacillus brevis, Lactobacillus casei, Lactobacillus coryniformis, Lactobacillus collinoides, Lactobacillus lindneri, Lactobacillus rossiae, Lactobacillus parabuchneri, Lactobacillus paracollinoides, Lactobacillus perolens, Lactobacillus plantarum, Pectinatus cerevisiiphillus, Pectinatus frisingensis, Pectinatus haikarae, Pectinatus portalensis, Megasphaera cerevisiae,* and *Megasphaera elsdenii.*

11. The method according to any one of claims 7-10, wherein the foodstuff is a beverage.

12. The method according to any one of claims 7-11, wherein the foodstuff is beer.

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