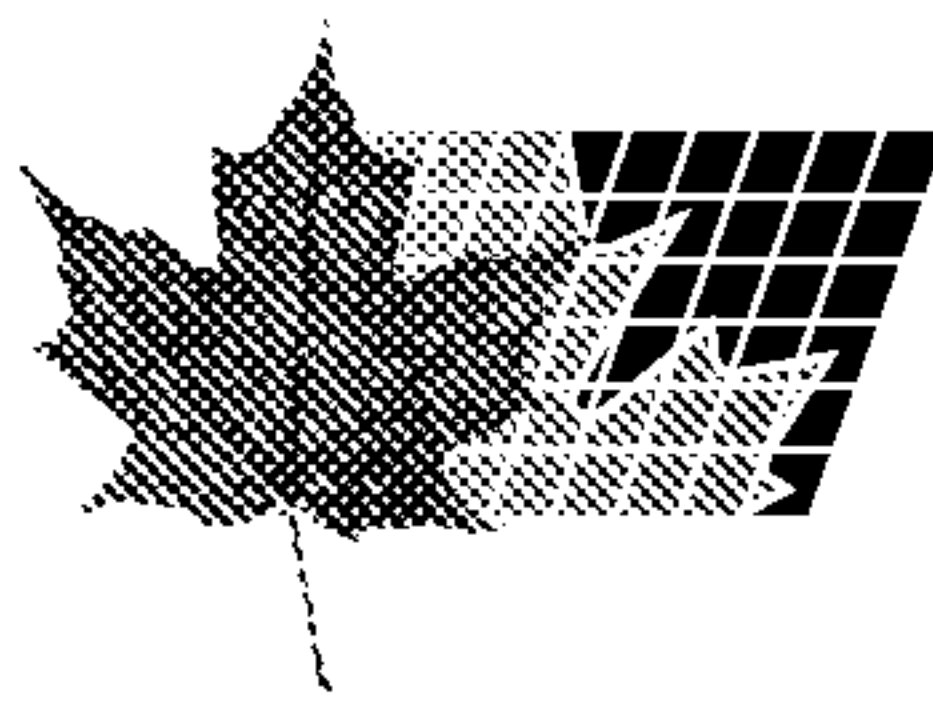




(72) ENGEL, STACIA R., US

(72) DESCENZO, RICHARD A., US

(72) MORENZONI, RICHARD A., US

(72) IRELAN, NANCY A., US

(71) E. & J. GALLO WINERY, US

(51) Int.Cl.<sup>6</sup> C12Q 1/68, C07H 21/04

(30) 1998/03/11 (09/037,990) US

(54) **DETECTION DES ORGANISMES LIES A LA FERMENTATION**

(54) **DETECTION OF FERMENTATION-RELATED  
MICROORGANISMS**

(57) L'invention concerne des séquences d'ADN uniques qui servent à identifier différents micro-organismes liés à la fermentation, par exemple ceux qui participent à des processus de fermentation. On peut utiliser ces séquences d'ADN uniques pour fournir des amorces polynucléotidiques pour l'analyse basée sur ACP et visant à détecter des micro-organismes liés à la fermentation. Les séquences d'ADN de la présente invention comprennent l'espaceur interne transcrit des régions géniques de l'ARN ribosomique de micro-organismes déterminés liés à la fermentation ainsi que des amorces oligonucléotidiques dérivées de ces régions et capable d'identifier un micro-organisme déterminé.

(57) Unique DNA sequences are provided which are useful in identifying different fermentation-related microorganisms, such as those involved in fermentations. These unique DNA sequences can be used to provide oligonucleotide primers in PCR based analysis for the identification of fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of particular fermentation-related microorganisms, as well as oligonucleotide primers which are derived from these regions which are capable of identifying the particular microorganism.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |           |                                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12Q 1/68, C07H 21/04</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/46405</b><br><b>(43) International Publication Date:</b> 16 September 1999 (16.09.99)                                                                                                                                                                                                                       |
| <b>(21) International Application Number:</b> PCT/US99/04251<br><b>(22) International Filing Date:</b> 11 March 1999 (11.03.99)<br><br><b>(30) Priority Data:</b><br>09/037,990                      11 March 1998 (11.03.98)                      US<br><br><b>(71) Applicant:</b> E. & J. GALLO WINERY [US/US]; 600 Yosemite Boulevard, Modesto, CA 95354 (US).<br><br><b>(72) Inventors:</b> ENGEL, Stacia, R.; 4063-A Elmo Loop, Modesto, CA 95356 (US). DESCENZO, Richard, A.; 3808 Lauring Avenue, Modesto, CA 95357 (US). MORENZONI, Richard, A.; 2800 Warwick Lane, Modesto, CA 95350 (US). IRELAN, Nancy, A.; 2028 Monte Carlo Court, Modesto, CA 95350 (US).<br><br><b>(74) Agents:</b> SWECKER, Robert, S. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US). |           | <b>(81) Designated States:</b> AU, BR, CA, CN, JP, NZ, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).<br><br><b>Published</b><br><i>With international search report.<br/>         Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| <b>(54) Title:</b> DETECTION OF FERMENTATION-RELATED MICROORGANISMS<br><br><b>(57) Abstract</b><br><br>Unique DNA sequences are provided which are useful in identifying different fermentation-related microorganisms, such as those involved in fermentations. These unique DNA sequences can be used to provide oligonucleotide primers in PCR based analysis for the identification of fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of particular fermentation-related microorganisms, as well as oligonucleotide primers which are derived from these regions which are capable of identifying the particular microorganism.                                                                 |           |                                                                                                                                                                                                                                                                                                                                                                    |

## **DETECTION OF FERMENTATION-RELATED MICROORGANISMS**

### **Field of the Invention**

The present invention relates to assays to detect fermentation-related microorganisms.

### **5     Description of the Related Art**

In traditional wine-making, the indigenous yeasts ferment the grape must. Most modern wine-makers, however, inoculate with a pure culture of a selected yeast strain to ensure a rapid, reliable and predictable fermentation. It is thought that indigenous yeasts are suppressed by the competitive effect of  
10     addition of a high-density monoculture, but some evidence suggests that indigenous yeast can still participate in the fermentation. A range of commercial yeasts with different wine-making characteristics is available, and a number of those yeast strains may be used in a single winery. Furthermore, unwanted microorganisms may be present which lead to spoilage. Therefore,  
15     there is a need for a rapid, simple and accurate method for identifying microorganisms in starter cultures and fermentations.

Past techniques for detecting and identifying fermentation-related microorganisms, especially yeast, include colony morphology, fermentation performance, sugar fermentation tests, tolerance to various stresses (e.g.,  
20     ethanol tolerance in brewing), phenotypes with functional relevance (e.g., flocculation in brewing), nutritional requirements (e.g., oxygen), and resistance and sensitivity levels of cycloheximide. These methods, however, have numerous disadvantages, including lengthy analysis periods, inability to differentiate, e.g., different strains of yeast, and lack of reproducibility.

Recent developments in molecular biology and protein chemistry have provided new methods for identifying microorganisms, including DNA restriction fragment length polymorphisms, protein electrophoresis patterns and chromosome fingerprinting. Such techniques have been used for identifying fermentation-related microorganisms. See, for example, Casey et al, *Journal of the American Society of Brewing Chemists*, 48(3):100-106, 1990; Degre et al, *American Journal of Enology and Viticulture*, 40(4):309-315, 1989; Guillamon et al, *Systematic and Applied Microbiology*, 19:122-132, 1992; Hoeben et al, *Current Genetics*, 10:371-379, 1986; Mozina et al, *Letters in Applied Microbiology*, 24(4):311-315, 1997; Paffetti et al, *Research Microbiology*, 146:587-594, 1995; Panchal et al, *Journal of the Institute of Brewing*, 93:325-327, 1987; Querol et al, *Systematic and Applied Microbiology*, 15:439-446, 1992; Vezinhet et al, *Applied Microbiology and Biotechnology*, 32:568-571, 1990; and Vezinhet et al, *American Journal of Enology and Viticulture*, 43(1):83-86, 1992.

Polymerase chain reaction (PCR)-based techniques have also been used to detect fermentation-related microorganisms. See, for example, DeBarros Lopes et al, *Applied and Environmental Microbiology*, 62(12):4514-4520, 1996; Fell, *Molecular Marine Biology and Biotechnology*, 2(3):174-180, 1993; Fell, *Journal of Industrial Microbiology*, 14(6):475-477, 1995; Ibeas et al, *Applied and Environmental Microbiology*, 62(3):998-1003, 1996; Lavallee et al, *American Journal of Enology and Viticulture*, 45(1):86-91, 1994; Lieckfeldt et al, *Journal of Basic Microbiology*, 33(6):413-425, 1993; and Ness et al, *J. Sci. Food Agric.*, 62:89-94, 1993.

Ribosomal genes are suitable for use as molecular probe targets because of their high copy number. Non-transcribed and transcribed spacer sequences associated with ribosomal genes are usually poorly conserved and, thus, are

advantageously used as target sequences for the detection of recent evolutionary divergence. Fungal rRNA genes are organized in units. Each unit encodes mature subunits of 18S, 5.8S, and 28S rRNA. The internal transcribed spacer (ITS) region lies between the 18S and 28S rRNA genes and contains two  
5 variable non-coding spacers (referred to as ITS1 and ITS2) and the 5.8S rRNA gene (White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al.; pages 315-322). In addition, the transcriptional units are separated by non-transcribed spacer sequences (NTSs). The ITS and NTS sequences are particularly suitable for the detection of different fungal pathogens.

10 Kumeda et al (*Applied and Environmental Microbiology*, 62(8):2947-2952, 1996) describes use of PCR to amplify ribosomal DNA internal transcribed spacers in order to differentiate species of *Aspergillus* Section *Flavi*. The ITS1-5.8S-ITS2 region was amplified using universal primers, and the PCR product analyzed by the principle of single-strand conformation  
15 polymorphism. In addition, Gardes et al (In: *Methods in Molecular Biology, Vol. 50:Species Diagnostics Protocols: PCR and Other Nucleic Acid Methods*, Ed. J.P. Clapp, Humana Press, Totowa, NJ, (1996) pp. 177-186) describes restriction fragment length polymorphism (RFLP) analysis of fungal ITS regions amplified by PCR.

20 The PCR amplification of fungal ITS has also been described using other than universal primers. These methods allow for more specificity in identifying classes of fungi, or particular species of fungi. Thus, Gardes and Bruns (*Molecular Ecology*, 2:113-118, 1993) identified ITS primers which allow differentiation of DNA from basidiomycetes against ascomycete DNA.  
25 Identification of specific species has been observed using PCR primers directed to unique sequences in the ITS1 and/or ITS2 regions of fungal pathogens. See, for example, Hamelin et al, *Applied and Environmental Microbiology*, 62(11):4026-4031, 1996; Mazzola et al, *Phytopathology*, 86(4):354-360, 1996;

O'Gorman et al, *Canadian Journal of Botany*, 72:342-346, 1994; and U.S. Patent No. 5,585,238 to Ligon et al.

The present invention addresses the problem of detecting and identifying fermentation-related microorganisms by PCR-based techniques.

5     **Summary of the Invention**

          The present invention is directed to the identification of different fermentation-related microorganisms, particularly those involved in the production of wine. The present invention provides DNA sequences which exhibit variability between different fermentation-related microorganisms. In particular, the present invention identifies regions of DNA sequence located in the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of various fermentation-related microorganisms. Primers derived from the ITS can be used in polymerase chain reaction (PCR) based diagnostic assays to determine the presence or absence of specific fermentation-related microorganisms, including those involved in the production of wine. The primers can also be used as molecular probes to detect the presence of target DNA.

          Thus, in one aspect, the present invention provides an isolated double stranded nucleic acid of the full length ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the DNA sequence is selected from among Sequence ID NOS: 13 to 36.

          In another aspect, the present invention provides an oligonucleotide primer for identification of a fermentation-related microorganism, wherein the primer is a divergent portion of the ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the oligonucleotide primer is selected from among Sequence ID NOS: 65 to 98. Furthermore, the oligonucleotide primers may be selected from among sequences which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98,

primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64. A pair of the foregoing oligonucleotide primers for use in the amplification-based detection of an ITS of a fermentation-related microorganism is also provided.

In yet another aspect, a method is provided for detection of a fermentation-related microorganism which comprises: (a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage; (b) amplifying a part of the ITS of the fermentation-related microorganism using the DNA as a template in a polymerase chain reaction with the aforementioned oligonucleotide primers; and (c) visualizing the amplified part of the ITS sequence to determine whether the fermentation-related microorganism is present.

In still another aspect, kits are provided which are useful in detecting fermentation-related microorganisms.

#### **Brief Description of the Sequences in the Sequence Listing:**

- |    |              |                                                                                                                        |
|----|--------------|------------------------------------------------------------------------------------------------------------------------|
| 20 | SEQ ID NO: 1 | DNA sequence for the internal transcribed spacer of <i>Saccharomyces cerevisiae</i> and <i>Saccharomyces bayanus</i> . |
|    | SEQ ID NO: 2 | DNA sequence for the internal transcribed spacer of <i>Saccharomyces ludwigii</i> .                                    |
|    | SEQ ID NO: 3 | DNA sequence for the internal transcribed spacer of <i>Dekkera bruxellensis</i> .                                      |
| 25 | SEQ ID NO: 4 | DNA sequence for the internal transcribed spacer of <i>Dekkera intermedia</i> .                                        |

- SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of *Botrytis cinerea*.
- SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of *Penicillium crustosum*.
- 5 SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of *Penicillium expansum*.
- SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of *Hanseniaspora guilliermondii*.
- SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of *Debaryomyces carsonii*.
- 10 SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of *Pichia anomala*.
- SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of *Pichia kluyveri*.
- 15 SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of *Candida krusei*.
- SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.
- SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.
- 20 SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes ludwigii*.
- SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomycodes ludwigii*.
- 25 SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.
- SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.
- SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.
- SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.
- SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.

|    |               |                                                                    |
|----|---------------|--------------------------------------------------------------------|
|    | SEQ ID NO: 22 | DNA sequence for the ITS2 of <i>Botrytis cinerea</i> .             |
|    | SEQ ID NO: 23 | DNA sequence for the ITS1 of <i>Penicillium crustosum</i> .        |
|    | SEQ ID NO: 24 | DNA sequence for the ITS2 of <i>Penicillium crustosum</i> .        |
|    | SEQ ID NO: 25 | DNA sequence for the ITS1 of <i>Penicillium expansum</i> .         |
| 5  | SEQ ID NO: 26 | DNA sequence for the ITS2 of <i>Penicillium expansum</i> .         |
|    | SEQ ID NO: 27 | DNA sequence for the ITS1 of <i>Hanseniaspora guilliermondii</i> . |
|    | SEQ ID NO: 28 | DNA sequence for the ITS2 of <i>Hanseniaspora guilliermondii</i> . |
| 10 | SEQ ID NO: 29 | DNA sequence for the ITS1 of <i>Debaryomyces carsonii</i> .        |
|    | SEQ ID NO: 30 | DNA sequence for the ITS2 of <i>Debaryomyces carsonii</i> .        |
|    | SEQ ID NO: 31 | DNA sequence for the ITS1 of <i>Pichia anomala</i> .               |
|    | SEQ ID NO: 32 | DNA sequence for the ITS2 of <i>Pichia anomala</i> .               |
|    | SEQ ID NO: 33 | DNA sequence for the ITS1 of <i>Pichia kluyveri</i> .              |
| 15 | SEQ ID NO: 34 | DNA sequence for the ITS2 of <i>Pichia kluyveri</i> .              |
|    | SEQ ID NO: 35 | DNA sequence for the ITS1 of <i>Candida krusei</i> .               |
|    | SEQ ID NO: 36 | DNA sequence for the ITS2 of <i>Candida krusei</i> .               |
|    | SEQ ID NO: 37 | Oligonucleotide Sequence SXUITS1a.                                 |
|    | SEQ ID NO: 38 | Oligonucleotide Sequence SXLITS2a.                                 |
| 20 | SEQ ID NO: 39 | Oligonucleotide Sequence SXUITS1b.                                 |
|    | SEQ ID NO: 40 | Oligonucleotide Sequence SXLITS2b.                                 |
|    | SEQ ID NO: 41 | Oligonucleotide Sequence SLUITS1a.                                 |
|    | SEQ ID NO: 42 | Oligonucleotide Sequence SLLITS2a.                                 |
|    | SEQ ID NO: 43 | Oligonucleotide Sequence SLUITS1b.                                 |
| 25 | SEQ ID NO: 44 | Oligonucleotide Sequence SLLITS2b.                                 |
|    | SEQ ID NO: 45 | Oligonucleotide Sequence BRUITS1a.                                 |
|    | SEQ ID NO: 46 | Oligonucleotide Sequence BRLITS2.                                  |
|    | SEQ ID NO: 47 | Oligonucleotide Sequence BRUITS1b.                                 |
|    | SEQ ID NO: 48 | Oligonucleotide Sequence BCUITS1a.                                 |

|    |               |                                    |
|----|---------------|------------------------------------|
|    | SEQ ID NO: 49 | Oligonucleotide Sequence BCLITS2.  |
|    | SEQ ID NO: 50 | Oligonucleotide Sequence BCUITS1b. |
|    | SEQ ID NO: 51 | Oligonucleotide Sequence PXUITS1a. |
|    | SEQ ID NO: 52 | Oligonucleotide Sequence PXLITS2a. |
| 5  | SEQ ID NO: 53 | Oligonucleotide Sequence PXUITS1b. |
|    | SEQ ID NO: 54 | Oligonucleotide Sequence PXLITS2b. |
|    | SEQ ID NO: 55 | Oligonucleotide Sequence HGUITS1.  |
|    | SEQ ID NO: 56 | Oligonucleotide Sequence HGLITS2.  |
|    | SEQ ID NO: 57 | Oligonucleotide Sequence DXLITS2.  |
| 10 | SEQ ID NO: 58 | Oligonucleotide Sequence PAUITS1.  |
|    | SEQ ID NO: 59 | Oligonucleotide Sequence PALITS2.  |
|    | SEQ ID NO: 60 | Oligonucleotide Sequence PKLITS2.  |
|    | SEQ ID NO: 61 | Oligonucleotide Sequence CKUITS1.  |
|    | SEQ ID NO: 62 | Oligonucleotide Sequence CKLITS2.  |
| 15 | SEQ ID NO: 63 | Oligonucleotide Sequence SXU99.    |
|    | SEQ ID NO: 64 | Oligonucleotide Sequence SXL658.   |
|    | SEQ ID NO: 65 | Oligonucleotide Sequence SXU102.   |
|    | SEQ ID NO: 66 | Oligonucleotide Sequence SXL661.   |
|    | SEQ ID NO: 67 | Oligonucleotide Sequence SLU85.    |
| 20 | SEQ ID NO: 68 | Oligonucleotide Sequence SLL635.   |
|    | SEQ ID NO: 69 | Oligonucleotide Sequence SLU88.    |
|    | SEQ ID NO: 70 | Oligonucleotide Sequence SLL636.   |
|    | SEQ ID NO: 71 | Oligonucleotide Sequence SLU136.   |
|    | SEQ ID NO: 72 | Oligonucleotide Sequence SLL634.   |
| 25 | SEQ ID NO: 73 | Oligonucleotide Sequence BRU53A.   |
|    | SEQ ID NO: 74 | Oligonucleotide Sequence BRU53B.   |
|    | SEQ ID NO: 75 | Oligonucleotide Sequence BRU77.    |
|    | SEQ ID NO: 76 | Oligonucleotide Sequence BRL339.   |
|    | SEQ ID NO: 77 | Oligonucleotide Sequence BRL367.   |

|    |               |                                  |
|----|---------------|----------------------------------|
|    | SEQ ID NO: 78 | Oligonucleotide Sequence BRL390. |
|    | SEQ ID NO: 79 | Oligonucleotide Sequence BCU136. |
|    | SEQ ID NO: 80 | Oligonucleotide Sequence BCL393. |
|    | SEQ ID NO: 81 | Oligonucleotide Sequence BCU142. |
| 5  | SEQ ID NO: 82 | Oligonucleotide Sequence PXU87.  |
|    | SEQ ID NO: 83 | Oligonucleotide Sequence PXL495. |
|    | SEQ ID NO: 84 | Oligonucleotide Sequence PXU86.  |
|    | SEQ ID NO: 85 | Oligonucleotide Sequence PXL482. |
|    | SEQ ID NO: 86 | Oligonucleotide Sequence PXL491. |
| 10 | SEQ ID NO: 87 | Oligonucleotide Sequence HGU193. |
|    | SEQ ID NO: 88 | Oligonucleotide Sequence HGU231. |
|    | SEQ ID NO: 89 | Oligonucleotide Sequence HGL601. |
|    | SEQ ID NO: 90 | Oligonucleotide Sequence DXL447. |
|    | SEQ ID NO: 91 | Oligonucleotide Sequence DXL526. |
| 15 | SEQ ID NO: 92 | Oligonucleotide Sequence PAU133. |
|    | SEQ ID NO: 93 | Oligonucleotide Sequence PAL451. |
|    | SEQ ID NO: 94 | Oligonucleotide Sequence PKL356. |
|    | SEQ ID NO: 95 | Oligonucleotide Sequence CKU104. |
|    | SEQ ID NO: 96 | Oligonucleotide Sequence CKL354. |
| 20 | SEQ ID NO: 97 | Oligonucleotide Sequence ITS5.   |
|    | SEQ ID NO: 98 | Oligonucleotide Sequence ITS4.   |

### **Detailed Description Of The Invention**

The present invention provides unique DNA sequences which are useful in identifying fermentation-related microorganisms. These unique DNA sequences can be used as primers in PCR-based analysis for the identification of fermentation-related microorganisms, or as molecular probes to detect the presence of DNA from fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS)

of the ribosomal RNA gene regions of specific fermentation-related microorganisms, as well as primers that are derived from these regions which are capable of identifying the particular microorganism.

5 The DNA sequences of the invention are from the ITS of the ribosomal RNA gene region of fermentation-related microorganisms. However, the present invention is not limited to detecting the presence of the microorganisms in fermentation operations, *i.e.*, the invention can be used to detect the presence of such microorganisms from any source. There is variability in the ITS DNA sequences from different microorganisms. The ITS sequences can be aligned  
10 and compared. Primers can be designed based on regions within the ITS regions that contain the greatest differences in sequence among the fermentation-related microorganisms. The sequences and primers based on these sequences can be used to identify specific microorganisms.

DNA sequences of particular interest include ITS DNA sequences from  
15 *Saccharomyces* sp., especially *Saccharomyces cerevisiae* and *Saccharomyces bayanus*; *Saccharomycodes* sp., especially *Saccharomycodes ludwigii*; *Dekkera* sp., especially *Dekkera bruxellensis* and *Dekkera intermedia*; *Botrytis* sp., especially *Botrytis cinerea*; *Penicillium* sp., especially *Penicillium crustosum* and *Penicillium expansum*; *Hanseniaspora* sp., especially *Hanseniaspora*  
20 *guilliermondii*; *Debaryomyces* sp., especially *Debaryomyces carsonii*; *Pichia* sp., especially *Pichia anomala* and *Pichia kluyveri*; and *Candida* sp., especially *Candida krusei*. The ITS DNA sequences, as well as primers of interest, are set forth in SEQUENCE ID NOS: 1-100. The sequences are useful in PCR-based identification of fermentation-related microorganisms.

25 Methods for use of the primer sequences of the invention in PCR analysis are well known in the art. For example, see U.S. Patent Nos. 4,683,195; 4,683,202 and 5,585,238, the contents of all of which are hereby incorporated by reference.

The primer sequences of the invention can also be used as molecular probes to detect the presence of target DNA. The  $T_m$  for the primers ranges from about 48-58° C at 50 mM salt. The hybridization temperature is approximately 5-10° C below the melting temperature. Thus, the primers are hybridized to target DNA typically at a temperature ranging from about 43-55° C. Final wash conditions generally range from about 45-55° C at about 36 mM salt concentration. Specific hybridization as used herein means the use of a final high stringency wash in about 0.2X SSPE (salt concentration of about 36 mM) at a temperature appropriate for the particular primer. 1X SSPE contains 10 mM  $\text{NaH}_2\text{PO}_4$ , 180 mM NaCl, and 1 mM EDTA, at pH 7.4.

The ITS DNA sequences of the present invention can be cloned from fermentation-related microorganisms by methods known in the art. In general, the methods for the isolation of DNA from microorganism isolates are known. See, Raeder & Broda (1985) *Letters in Applied Microbiology* 2:17-20; Lee et al. (1990) *Fungal Genetics Newsletter* 35:23-24; and Lee and Taylor (1990) In: *PCR Protocols: A Guide to Methods and Applications*, Innes et al. (Eds.); pages 282-287; the contents of all of which are hereby incorporated by reference.

Alternatively, the ITS regions of interest can be identified by PCR amplification. Primers to amplify the entire ITS region can be synthesized according to White et al. (1990; In PCR Protocols; Eds.: Innes et al., pages 315-322, the contents of which are hereby incorporated by reference).

The ITS sequences were determined and the sequences were compared to locate divergences which might be useful to test in PCR to distinguish the different fermentation-related microorganisms. The sequences of the ITS regions which were determined are shown as Sequence ID NOS: 1 to 12. The DNA sequences for the ITS1 and ITS2 regions are shown as Sequence ID NOS: 13 to 36. From the identification of divergences, numerous primers were synthesized and tested in PCR-amplification. Purified microorganism

DNA and DNA isolated from fermentation cultures and colonies were used as templates for PCR-amplification. Thus, pairs of diagnostic primers were identified, *i.e.*, those which identified one particular fermentation-related microorganism species. Preferred primer combinations are able to distinguish  
5 between the different microorganisms in, for example, fermentation cultures. Primer sequences are set forth in Sequence ID NOS: 65 to 98, with flanking sequences depicted in Sequence ID NOS: 37 to 64. Thus, while oligonucleotide primers selected from among Sequence ID NOS: 65 to 98 are preferred, primers may also be used which contain at least 10 contiguous  
10 nucleotide bases from one of SEQ ID NOS: 65 to 98. Additionally, primers may be used which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases  
15 from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

The present invention provides numerous diagnostic primer combinations. The primers of the invention are designed based on sequence differences among the microorganism ITS regions. A minimum of one base  
20 pair difference between sequences can permit design of a discriminatory primer. In general, primers should have a theoretical melting temperature between about 55 °C to about 65 °C to achieve good sensitivity, and should be void of significant secondary structure and 3' overlaps between primer combinations. Primers are generally at least about 10 nucleotide bases, more  
25 preferably at least about 15 to about 20 nucleotide bases.

The oligonucleotide primers of the present invention are particularly useful in detecting microorganisms involved in fermentations, in particular, microorganisms selected from among *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces ludwigii*, *Dekkera bruxellensis*,

*Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*. However, the primers of the present invention can also be used to detect the presence of the foregoing  
5 microorganisms from any source.

The present invention also relates to the preparation of "kits" containing elements for detecting fermentation-related microorganisms. Such a kit may comprise a carrier to receive therein one or more containers, such as tubes or vials. Unlabeled or detectably labeled oligonucleotide primers may be  
10 contained in one or more of the containers. The oligonucleotide primers may be present in lyophilized form, or in an appropriate buffer. One or more enzymes or reagents for use in PCR reactions may be contained in one or more of the containers. The enzymes or reagents may be present alone or in admixture, and in lyophilized form or in appropriate buffers. The kit may also  
15 contain any other component necessary for carrying out the present invention, such as buffers, extraction agents, enzymes, pipettes, plates, nucleic acids, nucleoside triphosphates, filter paper, gel materials, transfer materials, and autoradiography supplies.

The examples below illustrate typical experimental protocols which can  
20 be used in the isolation of ITS sequences, the selection of suitable primer sequences, the testing of primers for selective and diagnostic efficacy, and the use of such primers to detect the presence of a fermentation-related microorganism. Such examples are provided by way of illustration and not by way of limitation.

## Examples

### Example 1

#### Culture of Yeast and Fungal Isolates

5        Viable isolates of *Botrytis cinerea*, *Penicillium crustosum*, and  
      *Penicillium expansum* were obtained from the American Type Culture  
Collection (ATCC). Fungi were grown in 40 ml of Malt Yeast Extract Broth  
in 250 ml flasks inoculated with mycelial fragments from two-week-old  
cultures grown on Malt Yeast Extract Agar (MYEA). Liquid cultures were  
incubated at room temperature for 14 days without shaking. Malt Yeast  
10    Extract Agar plates were inoculated with mycelia and grown for 2 weeks.  
      Viable isolates of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*,  
*Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera*  
*anomala*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia*  
*anomala*, *Pichia kluyveri*, and *Candida krusei* were obtained from the ATCC or  
15    from the Gallo Sonoma Winery (see Table 1). Yeasts were grown on any of  
several media of choice.

### Example 2

#### Amplification and Sequencing of the Internal Transcribed Spacer (ITS) Regions

      The internal transcribed spacer region was amplified from the different  
20    isolates directly from the fungal mycelium or the yeast colony using ITS5  
(5'-GGAAGTAAAAGTCGTAACAAGG-3'; SEQ ID NO: 99) and ITS4  
(5'-TCCTCCGCTTATTGATATGC-3'; SEQ ID NO: 100). A sterile pipette  
tip was used to scrape a small amount of mycelia or colony off of the plate and  
deposited into a 250- $\mu$ l microcentrifuge tube containing 5  $\mu$ l each of GeneAmp®  
25    10X PCR Buffer II and MgCl<sub>2</sub> solution (PE Applied Biosystems, Foster City,  
CA; part no. N808-0161), 0.2 mM each of dATP, dCTP, dGTP, and dTTP

(GeneAmp<sup>®</sup> dNTPs; PE Applied Biosystems, Foster City, CA; part no. N808-0007), approximately 25 pmole/ $\mu$ l each of ITS5 and ITS4, and 2.5 Units AmpliTaq<sup>®</sup> DNA polymerase (PE Applied Biosystems; part no. N808-0161). Reactions were run for 35 cycles of 30 s at 94 °C, 40 s at 58 °C, and 2 min at  
5 72 °C, followed by a final elongation step at 72 °C for 10 min, on a Perkin Elmer GeneAmp<sup>®</sup> PCR System 9600 (PE Applied Biosystems). PCR products were purified using QIAquick<sup>®</sup> PCR Purification Kits (Qiagen Inc., Santa Clarita, CA) to remove any excess primers, nucleotides, and polymerases. Five microliters of the purified PCR products were run on a 1.2% agarose gel  
10 with 5  $\mu$ l of pGEM<sup>-</sup>-3Zf(+) double-stranded DNA Control Template (0.2 g/L, PE Applied Biosystems) to approximate concentrations. All products were sequenced using the primers ITS5 and ITS4 (see sequences above; White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al. pp. 315-322). Sequencing was performed on an PE Applied Biosystems 377 Automated DNA Sequencer<sup>®</sup>  
15 using ABI PRISM<sup>™</sup> Dye Terminator Cycle Sequencing Ready Reaction Kits<sup>®</sup> (PE Applied Biosystems; part no. 402079). Cycle sequencing products were run over Centri-Sep<sup>®</sup> spin columns (Princeton Separations, Inc., Adelphia, NJ) to remove excess primers, dye-labeled terminators, nucleotides, and polymerases before being run on the automated sequencer.

20

### Example 3

#### Selection of Species-Specific Primers

The ITS sequences of the *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera anomala*, *Botrytis cinerea*, *Penicillium crustosum*,  
25 *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei* isolates were aligned and

primers were designed using Oligo 5.0 (National Biosciences, Inc., Plymouth, MN) in regions of maximum sequence difference between the target species.

#### Example 4

##### Primer Synthesis

5            Primers were synthesized on an Applied Biosystems 394 DNA/RNA Synthesizer<sup>®</sup> using phosphoramidite chemistry.

#### Example 5

##### Verification of Primer Specificity to Target Species

10           Different annealing temperatures were tested to determine the optimal temperature for PCR for individual primers. In cases with multiple species-specific primers, different primer combinations were used to determine the best primer combination and annealing temperature to amplify a single species-specific DNA fragment. Species-specific amplification products were produced from primers designed from the ITS region between the 18S and 28S ribosomal  
15           DNA subunits of each fungal strain of interest.

#### Example 6

##### Utilization of ITS sequences as diagnostic probes to hybridize with target DNA

1. Put chosen concentration of DNA sample in 100  $\mu$ l of TE, pH 7.0.
2. Add 0.1 volume [10  $\mu$ l] of 3.0 M NaOH, vortex to mix and incubate  
20           at  
65° C for 20 min to destroy the RNA and denature the DNA.
3. Spin down condensation. Allow samples to cool to room temp.  
Neutralize by adding 1.0 volume [110  $\mu$ l] of 2M ammonium acetate, pH 7.0,

vortex to mix. Spin down to remove solution off of cap. Refrigerate until slot blot apparatus is ready.

4. Apply to slot-blot apparatus according to manufacturers protocol; about 220  $\mu$ l to slot blot.

5           5. Label ITS sequence probe according to kit manufacturer's recommendation.

6. Prehybridize blots in 1.0% BSA; 1mM EDTA, 0.5 M NaHPO<sub>4</sub>, pH 7.2, 7.0% sodium dodecyl sulfate for a minimum of 2 hr prior to adding the probe, and then hybridized for 16 hr at 45° C. Initial washes consist of two 30-  
10 min washes in 1X SSPE/0.1% SDS at 50° C. Transfer blots to a plastic tray and wash in 1X SSPE for 1 hr, at 50° C with shaking. The final wash should consist of 15 min at 50° C in 0.2X SSPE.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that  
15 various changes and modifications can be made therein without departing from the spirit and scope thereof.

Table 1  
Sources of test isolates

|    | Species name                        | ID number | Source                       |
|----|-------------------------------------|-----------|------------------------------|
| 5  | <i>Saccharomyces cerevisiae</i>     | GS061     | Gallo of Sonoma <sup>1</sup> |
|    | <i>Saccharomyces cerevisiae</i>     | GS117     | Gallo of Sonoma              |
|    | <i>Saccharomyces cerevisiae</i>     | 4127      | ATCC <sup>2</sup>            |
|    | <i>Saccharomyces bayanus</i>        | 13056     | ATCC                         |
|    | <i>Saccharomyces ludwigii</i>       | 34085     | ATCC                         |
| 10 | <i>Saccharomyces ludwigii</i>       | 44299     | ATCC                         |
|    | <i>Dekkera bruxellensis</i>         | Y153      | Gallo of Sonoma              |
|    | <i>Dekkera bruxellensis</i>         | Y207      | Gallo of Sonoma              |
|    | <i>Dekkera bruxellensis</i>         | 10560     | ATCC                         |
|    | <i>Dekkera intermedia</i>           | 34448     | ATCC                         |
| 15 | <i>Dekkera anomala</i>              | 10559     | ATCC                         |
|    | <i>Botrytis cinerea</i>             | 20599     | ATCC                         |
|    | <i>Penicillium crustosum</i>        | 58616     | ATCC                         |
|    | <i>Penicillium expansum</i>         | 28885     | ATCC                         |
|    | <i>Hanseniaspora guilliermondii</i> | GS014     | Gallo of Sonoma              |
| 20 | <i>Hanseniaspora guilliermondii</i> | GS057     | Gallo of Sonoma              |
|    | <i>Hanseniaspora guilliermondii</i> | 66166     | ATCC                         |
|    | <i>Debaryomyces carsonii</i>        | Y443      | Gallo of Sonoma              |
|    | <i>Debaryomyces carsonii</i>        | Y448      | Gallo of Sonoma              |
|    | <i>Debaryomyces carsonii</i>        | 24214     | ATCC                         |
| 25 | <i>Pichia anomala</i>               | GS085     | Gallo of Sonoma              |
|    | <i>Pichia anomala</i>               | 34080     | ATCC                         |
|    | <i>Pichia kluyveri</i>              | GS070     | Gallo of Sonoma              |
|    | <i>Pichia kluyveri</i>              | 64303     | ATCC                         |
|    | <i>Candida krusei</i>               | GS076     | Gallo of Sonoma              |
| 30 | <i>Candida krusei</i>               | GS096     | Gallo of Sonoma              |
|    | <i>Candida krusei</i>               | GS108     | Gallo of Sonoma              |

<sup>1</sup>Gallo of Sonoma Winery, Healdsburg, CA, USA

<sup>2</sup>American Type Culture Collection, Rockville, MD, USA

Table 2

|    | Target Organism                 | Primer Name | Primer Sequence                                |
|----|---------------------------------|-------------|------------------------------------------------|
|    | <i>Saccharomyces</i> sp.        | SXU99       | 5'-CTTTTACTGGGCAAGAAGAC-3'<br>(SEQ ID NO: 65)  |
|    | <i>Saccharomyces</i> sp.        | SXL658      | 5'-AAGCACGCAGAGAAACC-3'<br>(SEQ ID NO: 66)     |
| 5  | <i>Saccharomyces</i> sp.        | SXU102      | 5'-TTACTGGGCAAGAAGACAAG-3'<br>(SEQ ID NO: 67)  |
|    | <i>Saccharomyces</i> sp.        | SXL661      | 5'-CTCAAGCACGCAGAGAA-3'<br>(SEQ ID NO: 68)     |
|    | <i>Saccharomycodes ludwigii</i> | SLU85       | 5'-GACTTTTCTTGGGGAGAG-3'<br>(SEQ ID NO: 69)    |
| 10 | <i>Saccharomycodes ludwigii</i> | SLL635      | 5'-TCACTAGTTGGGATAAACCT-3'<br>(SEQ ID NO: 70)  |
|    | <i>Saccharomycodes ludwigii</i> | SLU88       | 5'-TTTTCTTGGGGAGAGG-3'<br>(SEQ ID NO: 71)      |
|    | <i>Saccharomycodes ludwigii</i> | SLL636      | 5'-ATCACTAGTTGGGATAAACCT-3'<br>(SEQ ID NO: 72) |
| 15 | <i>Saccharomycodes ludwigii</i> | SLU136      | 5'-CGGCTAGTAGTTGATGATT-3'<br>(SEQ ID NO: 73)   |
|    | <i>Saccharomycodes ludwigii</i> | SLL634      | 5'-CACTAGTTGGGATAAACCTA-3'<br>(SEQ ID NO: 74)  |
|    | <i>Dekkera</i> sp.              | BRU53A      | 5'-ATTACAGGATGCTGGGC-3'<br>(SEQ ID NO: 75)     |
| 20 | <i>Dekkera</i> sp.              | BRU53B      | 5'-ATTACAGGATGCTGGG-3'<br>(SEQ ID NO: 76)      |
|    | <i>Dekkera</i> sp.              | BRU77       | 5'-CGTGCAGACACGTGGAT-3'<br>(SEQ ID NO: 77)     |
|    | <i>Dekkera</i> sp.              | BRL339      | 5'-CTTTGAAGAAAACACCCTC-3'<br>(SEQ ID NO: 78)   |
|    | <i>Dekkera</i> sp.              | BRL367      | 5'-AATCATTATCCCCTCACTC-3'<br>(SEQ ID NO: 79)   |

|    | Target Organism                     | Primer Name | Primer Sequence                                |
|----|-------------------------------------|-------------|------------------------------------------------|
|    | <i>Dekkera</i> sp.                  | BRL390      | 5'-TAATGAACGGCCGAAAC-3'<br>(SEQ ID NO: 80)     |
|    | <i>Botrytis cinerea</i>             | BCU136      | 5'-TTGTATGCTCGCCAGAG-3'<br>(SEQ ID NO: 81)     |
|    | <i>Botrytis cinerea</i>             | BCL393      | 5'-GCCTGCCATTACTGACA-3'<br>(SEQ ID NO: 82)     |
|    | <i>Botrytis cinerea</i>             | BCU142      | 5'-GCTCGCCAGAGAATACC-3'<br>(SEQ ID NO: 83)     |
| 5  | <i>Penicillium</i> sp.              | PXU87       | 5'-ACCCGTCTTTATTTTACCTT-3'<br>(SEQ ID NO: 84)  |
|    | <i>Penicillium</i> sp.              | PXL495      | 5'-CTACAGAGCGGGTGACA-3'<br>(SEQ ID NO: 85)     |
|    | <i>Penicillium</i> sp.              | PXU86       | 5'-CACCCGTGTTTATTTTACCT-3'<br>(SEQ ID NO: 86)  |
|    | <i>Penicillium</i> sp.              | PXL482      | 5'-CAAAGCCCCATACGC-3'<br>(SEQ ID NO: 87)       |
|    | <i>Penicillium</i> sp.              | PXL491      | 5'-AGCGGGTGACAAAGC-3'<br>(SEQ ID NO: 88)       |
| 10 | <i>Hanseniaspora guilliermondii</i> | HGU193      | 5'-CAACGTTTACACACATTGG-3'<br>(SEQ ID NO: 89)   |
|    | <i>Hanseniaspora guilliermondii</i> | HGU231      | 5'-AATTCTTTCTGCTTTGAATCG-3'<br>(SEQ ID NO: 90) |
| 15 | <i>Hanseniaspora guilliermondii</i> | HGL601      | 5'-CAGCGTCTCCAAAGAAGCTAA-3'<br>(SEQ ID NO: 91) |
|    | <i>Debaryomyces carsonii</i>        | DXL447      | 5'-GCAAACGCCTAGTTCGACTAA-3'<br>(SEQ ID NO: 92) |
|    | <i>Debaryomyces carsonii</i>        | DXL526      | 5'-ATTCAACGAGTTGGATAAACC-3'<br>(SEQ ID NO: 93) |
| 20 | <i>Pichia anomala</i>               | PAU133      | 5'-GGCTTACTGCCCAAAGGTC-3'<br>(SEQ ID NO: 94)   |
|    | <i>Pichia anomala</i>               | PAL451      | 5'-TGCTTATTAGTACACTCTTGC-3'<br>(SEQ ID NO: 95) |

WO 99/46405

PCT/US99/04251

| Target Organism        | Primer Name | Primer Sequence                                |
|------------------------|-------------|------------------------------------------------|
| <i>Pichia kluyveri</i> | PKL356      | 5'-GTTTAGTTCACTTCGTCCACG-3'<br>(SEQ ID NO: 96) |
| <i>Candida krusei</i>  | CKU104      | 5'-CCTAAAATGTGGAATATAGCA-3'<br>(SEQ ID NO: 97) |
| <i>Candida krusei</i>  | CKL354      | 5'-ACGCTCTTTACACGTCGTC-3'<br>(SEQ ID NO: 98)   |

Table 3

## ITS-derived Diagnostic PCR Primers

|    | Target Organism                 | 5' Primer | 3' Primer | Approximate Size of<br>PCR Product (bp) |
|----|---------------------------------|-----------|-----------|-----------------------------------------|
| 5  | <i>Saccharomyces sp.</i>        | SXU99     | ITS4      | 750                                     |
|    | <i>Saccharomyces sp.</i>        | SXU102    | ITS4      | 750                                     |
|    | <i>Saccharomyces sp.</i>        | ITS5      | SXL658    | 695                                     |
|    | <i>Saccharomyces sp.</i>        | ITS5      | SXL661    | 695                                     |
|    | <i>Saccharomyces sp.</i>        | SXU99     | SXL658    | 560                                     |
| 10 | <i>Saccharomyces sp.</i>        | SXU99     | SXL661    | 560                                     |
|    | <i>Saccharomyces sp.</i>        | SXU102    | SXL658    | 560                                     |
|    | <i>Saccharomyces sp.</i>        | SXU102    | SXL661    | 560                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU85     | ITS4      | 660                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU88     | ITS4      | 660                                     |
| 15 | <i>Saccharomycodes ludwigii</i> | SLU136    | ITS4      | 610                                     |
|    | <i>Saccharomycodes ludwigii</i> | ITS5      | SLL635    | 670                                     |
|    | <i>Saccharomycodes ludwigii</i> | ITS5      | SLL636    | 670                                     |
|    | <i>Saccharomycodes ludwigii</i> | ITS5      | SLL634    | 670                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU85     | SLL635    | 565                                     |
| 20 | <i>Saccharomycodes ludwigii</i> | SLU85     | SLL636    | 565                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU85     | SLL634    | 565                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU88     | SLL635    | 565                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU88     | SLL636    | 565                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU88     | SLL634    | 565                                     |
| 25 | <i>Saccharomycodes ludwigii</i> | SLU136    | SLL635    | 500                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU136    | SLL636    | 500                                     |
|    | <i>Saccharomycodes ludwigii</i> | SLU136    | SLL634    | 500                                     |
|    | <i>Dekkera sp.</i>              | BRU53A    | ITS4      | 450                                     |
|    | <i>Dekkera sp.</i>              | BRU53B    | ITS4      | 450                                     |
| 30 | <i>Dekkera sp.</i>              | BRU77     | ITS4      | 450                                     |
|    | <i>Dekkera sp.</i>              | ITS5      | BRL339    | 375                                     |
|    | <i>Dekkera bruxellensis</i>     | ITS5      | BRL367    | 400                                     |
|    | <i>Dekkera sp.</i>              | ITS5      | BRL390    | 425                                     |
|    | <i>Dekkera sp.</i>              | BRU53A    | BRL339    | 290                                     |
| 35 | <i>Dekkera bruxellensis</i>     | BRU53A    | BRL367    | 320                                     |
|    | <i>Dekkera sp.</i>              | BRU53A    | BRL390    | 340                                     |

|    |                                     |        |        |     |
|----|-------------------------------------|--------|--------|-----|
|    | <i>Dekkera sp.</i>                  | BRU53B | BRL339 | 290 |
|    | <i>Dekkera bruxellensis</i>         | BRU53B | BRL367 | 320 |
|    | <i>Dekkera sp.</i>                  | BRU53B | BRL390 | 340 |
|    | <i>Dekkera sp.</i>                  | BRU77  | BRL339 | 260 |
| 5  | <i>Dekkera bruxellensis</i>         | BRU77  | BRL367 | 290 |
|    | <i>Dekkera sp.</i>                  | BRU77  | BRL390 | 310 |
|    | <i>Botrytis cinerea</i>             | BCU136 | ITS4   | 350 |
|    | <i>Botrytis cinerea</i>             | BCU142 | ITS4   | 345 |
|    | <i>Botrytis cinerea</i>             | ITS5   | BCL393 | 420 |
| 10 | <i>Botrytis cinerea</i>             | BCU136 | BCL393 | 270 |
|    | <i>Botrytis cinerea</i>             | BCU142 | BCL393 | 265 |
|    | <i>Penicillium sp.</i>              | PXU87  | ITS4   | 410 |
|    | <i>Penicillium sp.</i>              | PXU86  | ITS4   | 410 |
|    | <i>Penicillium sp.</i>              | ITS5   | PXL495 | 530 |
| 15 | <i>Penicillium sp.</i>              | ITS5   | PXL482 | 520 |
|    | <i>Penicillium sp.</i>              | ITS5   | PXL491 | 530 |
|    | <i>Penicillium sp.</i>              | PXU87  | PXL495 | 405 |
|    | <i>Penicillium sp.</i>              | PXU87  | PXL482 | 395 |
|    | <i>Penicillium sp.</i>              | PXU87  | PXL491 | 405 |
| 20 | <i>Hanseniaspora guilliermondii</i> | HGU193 | ITS4   | 530 |
|    | <i>Hanseniaspora guilliermondii</i> | HGU231 | ITS4   | 490 |
|    | <i>Hanseniaspora guilliermondii</i> | ITS5   | HGL601 | 630 |
| 25 | <i>Hanseniaspora guilliermondii</i> | HGU193 | HGL601 | 420 |
|    | <i>Hanseniaspora guilliermondii</i> | HGU231 | HGL601 | 380 |
| 30 | <i>Debaryomyces carsonii</i>        | ITS5   | DXL447 | 480 |
|    | <i>Debaryomyces carsonii</i>        | ITS5   | DXL526 | 560 |
|    | <i>Pichia anomala</i>               | PAU133 | ITS4   | 505 |
|    | <i>Pichia anomala</i>               | ITS5   | PAL451 | 480 |
|    | <i>Pichia anomala</i>               | PAU133 | PAL451 | 320 |
| 35 | <i>Pichia kluyveri</i>              | ITS5   | PKL356 | 390 |
|    | <i>Candida krusei</i>               | CKU104 | ITS4   | 440 |
|    | <i>Candida krusei</i>               | ITS5   | CKL354 | 385 |

**Sequence Listing**

SEQ ID NO: 1      DNA sequence for the internal transcribed spacer of  
*Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5'      AAGAAATTTAATAATTTTGAAAATGGATTTTTTTTGTTTT  
5      GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA  
GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC  
GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA  
AACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATT  
AAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT  
10      CTTTGCAACTTTTTCTTTGGGCATTTCGAGCAATCGGGGC  
CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA  
AATTTTTGTCAAAAACAAGAATTTTCGTAACCTGGAAATT  
TAAAATATTAAAACTTTCAACAACGGATCTCTTGGTT  
CTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTA  
15      ATGTGAATTGCAGAATCCCGTGAATCATCGAATCTTTGA  
ACGCACATTGCGCCCCTTGGTATTCCAGGGGGGCATGCCT  
GTTTGAGCGTCATTTCTTCTCAAACATTCTGTTTGGTA  
GTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGC  
CTTTTCATTGGATGTTTTTTTTTCCAAAGAGAGGTTTCTC  
20      TGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGG  
TTTTACCAACTGCGGCTAATCTTTTTTTATACTGAGCGT  
ATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGA  
ACAATGTTCTTAAAGT 3'

SEQ ID NO: 2      DNA sequence for the internal transcribed spacer of  
25      *Saccharomyces ludwigii*.

5'      AAGAAAAAACTGTTTATAAACAGACGGTAGACTTTTC  
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC  
TGCGCGGCTAGTAGTTGATGATTTTGTTATTATCCGAGA  
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG  
30      GAGTACTTTTTTTAATGTATTTCTTCCTTGGACGAGCAA  
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA  
TTTAAATTTAAAATAATTCAAAATATATCATTTCTTTTA  
TTAGGAATATAAAAATTTTAAAACCTTCAACAACGGATC  
TCTTGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCG  
35      ATAAGTAATGTGAATTGCAGATTTTCGTGAATCATTGAA  
TTTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGG  
CATGCCTGTTTGAGCGTCATTTCTTCTCAAAGAGTTT  
TTTTATTCTTTTGGTTGTGAGTGATACTTTCTTTTAC

AGGGAAGGGGTAACTTGAAATTGTTGCCTAGCAAAGA  
 AGAATTTTGATTGAAATTTCTTGTTTATTACTATTAGGT  
 TTATCCCAACTAGTGATTATTGAGAGTTTTTATTACAGA  
 GTCTTTTCACTTGCTATAATACTATTCTATAAGT 3'

5 SEQ ID NO: 3 DNA sequence for the internal transcribed spacer of  
*Dekkera bruxellensis*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
 AGCAAGGATAAAAATACATTAAATTTATTAGTTTAGTC  
 AAGAAAGAATTTTAAACTTTCAACAATGGATCTCTTGG  
 10 TTCTCGCGTCGATGAAGAGCGCAGCGGAATTGCGATAC  
 TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT  
 TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGGCAT  
 GCCTGTTTGAGCGTCATTTCTTCTCACTATTTAGTGGT  
 TATGAGATTACACGAGGGGTGTTTTCTTCAAAGGAAAGA  
 15 GGGGAGAGTGAGGGGATAATGATTTAAGGTTTCGGCCG  
 TTCATTATTTTTTTCTTCTCCCCCAGTTATCAAGT 3'

SEQ ID NO: 4 DNA sequence for the internal transcribed spacer of  
*Dekkera intermedia*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
 20 AGTAAGGATAAAAATACATTAAATTTATTAGTTTTAGT  
 CAAGAAAGAATTTTAAACTTTCAACAATGGATCTCTTG  
 GTTCTCGCGTCGATGAAGAGCGCAGCGAATTGCGATAC  
 TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT  
 TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGGCAT  
 25 GCCTGTTTGAGCGTCATTTCTTCTCACTATTTAGTGGT  
 TATGAGATTACACGAGGGGTGTTTTCTTCAAAGGAAAGA  
 GGGGAGAGTGAGGGGATAATGATTTAAGGTTTCGGCCG  
 TTCATTATTTTTTTCTTCTCCCCCAGTTATCAAGT 3'

30 SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of  
*Botrytis cinerea*.

5' CAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTG  
 TGTATTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG  
 CCTTGATGCTCGCCAGAGAATACCAAACTCTTTTTAT  
 TAATGTCGTCTGAGTACTATATAATAGTTAAAACTTTCA

5 ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC  
 AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG  
 TGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTTGG  
 TATTCCGGGGGGGCATGCCTGTTGAGCGTCATTTCAACC  
 CTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGTAATGG  
 CAGGCTCTAAAATCAGTGGCGGGCGCCGCTGGGTCCTGA  
 ACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTT  
 CTGCCAAAACCCAAATTTTTCTATGG 3'

10 SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of  
*Penicillium crustosum*.

5' CCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG  
 TTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAAC  
 GGCCGCCGGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC  
 CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT  
 15 GAGTGAAAATATAAATTATTTAAACTTTCAACAACGG  
 ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAA  
 TGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATC  
 GAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG  
 GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCC  
 20 GGCTTGTGTGTTGGGCCCCGTCCCCCGATCTCCGGGGGA  
 CGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCT  
 CGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGG  
 CCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGG 3'

25 SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of  
*Penicillium expansum*.

5' CCGAGTGAGGGCCCTTTGGGTCCAACCTCCCACCCGTGT  
 TTATTTACCTCGTTGCTTCGGCGGGCCCGCCTTAAC  
 CCGCCGGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG  
 AAGACACCCCCGAACCTCTGCCTGAAGATTGTCGTCTGA  
 30 GTGAAAATATAAATTATTTAAACTTTCAACAACGGATC  
 TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGC  
 GATACGTAATGTGAATTGCAAATTCAGTGAATCATCGA  
 GTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGG  
 GCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGG  
 35 CTTGTGTGTTGGGCCCCGTCTCCGATTCCGGGGGACGG  
 GCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGA  
 GCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCG  
 GCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACC

TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT  
CAATAAGCGGAGGAAA 3'

SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of  
*Hanseniaspora guilliermondii*.

5' 5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT  
CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT  
TAATTGCGCTGCTTTTTTTAAAGTGTCGCAGTAGAAGTAA  
TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG  
TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA  
10 AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT  
TATTATAATTTTTTAAACTAAACCAAATTCCTAACGGA  
AATTTTAAAATAATTTAAAACTTTCAACAACGGATCTCT  
TGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCGAT  
AAKTAATGTGAATTGCAGATACTCGTGAATCATTGAATT  
15 TTTGAACGCACATTGCGCCCTTGAGCATTCTCAAGGGCA  
TGCCTGTTTGAGCGTCATTTCTTCTCAAAGATAATTT  
TTTATTTTTTTGGTTGTGGGCGATACTCAGGGTTAGCTTG  
AAATTGAAGATTGTTTCAATCTTTTTTAATTCAACACTT  
AGCTTCTTTGGAGACGCTGTTCTCGCTGTGATGTATTTA  
20 TGAATTTATTCGTTTTACTTTACAAGGGAAATGGTAATG  
TACCTTAGGCAAAGGGTTGCTTTTAATATTCATCAAGT  
3'

SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of  
*Debaryomyces carsonii*.

25 5' CAGTATTCTTTTTGCCAGCGCTTAACTGCGCGGCGAAAT  
AAACCTTACACACAATGTTTTTTGTTATTACAGGAACTT  
TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG  
GTTTAAACTAACTTCAATTTATTTGAACTATTTTTCTTAT  
TGAAATGTCACCTTTGTTGATTAAATTCAAAAATCTTCA  
30 AAACCTTCAACAACGGATCTCTTGGTTCTCGCATCGATG  
AAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA  
GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCG  
CCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCA  
TTTCTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATAC  
35 TCTTAGTCGAACCTAGGCGTTTGCTTGAAATGTATCGGCA  
TGAGTGGTACTAGATTAGTGCTTCAGATTTTTCAATGTA  
TTAGGTTTATCCAACCTCGTTGAATAGTCTGATGGCAAGT

GTTTAGTAACTATGGCTCGGCCTAACAACAACAAACAA  
GT 3'

SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of  
*Pichia anomala*.

5 5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA  
CCTTACACACATTGTCTAGTTTTTTTTGAACTTTGCTTTGG  
GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA  
TTTTTTTAATGTTAAAACCTTTAACCAATAGTCATGAAA  
ATTTTAAACAAAAATTAATAATCTTCAAAACTTTCAACAA  
10 CGGATCTCTTGGTTCTCGCAACGATGAAGAACGCAGCG  
AAATGCGATACGTATTGTGAATTGCAGATTTTCGTGAAT  
CATCGAATCTTTGAACGCACATTGCACCCTCTGGTATTC  
CAGAGGGTATGCCTGTTTGAGCGTCATTTCTCTCTCAAA  
CCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTT  
15 AACTTGAAATATTGACTTAGCAAGAGTGTACTAATAAG  
CAGTCTTCTGAAATAATGTATTAGGTTCTTCCAACCTCG  
TTATATCAGCTAGGCAGGTTTAGAAGTATTTTAGGCTCG  
GCTTAACAACAATAAACTAAAAGT 3'

20 SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of  
*Pichia kluyveri*.

5' CTGTGATTTATATCTTATAACACATGCGTGAGCGCACCAA  
ACACCTAAAATTGTAATAATACCAGTCACTAAGTTTTAA  
CAAAACAAAACCTTTCAACAACGGATCTCTTGGTTCTCGC  
25 ATCGATGAAGAGCGCAGcGAAATGCGATACCTAGTGTG  
AATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCAC  
ATTGCGCCCCATGGTATTCCATGGGGCATGCCTGTCTGA  
GCGTCGTTTCCTTCTTGCGCAAGCAGAGTTGAGAACAG  
GCTATGCCTTTTTTCGAAATGGAACGTCGTGGACGAAGT  
30 GAACTAAACTTTTAGCACGCTTTGGCCGCCGAACCTTTTA  
ACTAAGC 3'

SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of  
*Candida krusei*.

5' CTGTGATTTACTACTACACTGCGTGAGCGGAACGAAAA  
CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA

5 CAAGAGAAATCTACGAAAAACAAACAAAACCTTTCAAC  
AACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAG  
CGAAATGCGATACCTAGTGTGAATTGCAGCCATCGTGA  
ATCATCGAGTTCTTGAACGCACATTGCGCCCCTCGGCAT  
TCCGGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTG  
CGCGTGCGCAGAGTTGGGGGAGCGGAGCGGACGACGTG  
TAAAGAGCGTCGGAGCTGCGACTCGCCTGAAAGGGAGC  
GAAGCTGGCCGAGCGAACTAGACTTTTTTTCAGGGACG  
10 CTTGGCGGCCGAGAGCGAGTGTTGCGAGACAACAAAAA  
GC 3'

SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae*  
and *Saccharomyces bayanus*.

5' AAGAAATTTAATAATTTTGAAAATGGATTTTTTTTGTTTT  
GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA  
15 GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC  
GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA  
AACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATT  
AAAACCGTTTCAATACACACACTGTGGAGTTTTTCATAT  
CTTTGCAACTTTTTCTTTGGGCATTTCGAGCAATCGGGGC  
20 CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA  
AATTTTTGTCAAAAACAAGAATTTTCGTAACCTGGAAATT  
TTAAAATATTAA 3'

SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae*  
and *Saccharomyces bayanus*.

25 5' CCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTT  
TGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGT  
TTTTTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGT  
ATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCG  
GCTAATCTTTTTTTATACTGAGCGTATTGGAACGTTATC  
30 GATAAGAAGAGAGCGTCTAGGCGAACAATGTTCTTAAA  
GT 3'

SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes*  
*ludwigii*.

5' AAGAAAAAACTGTTTATAAACAGACGGTAGACTTTTC  
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC  
TGCGCGGCTAGTAGTTGATGATTTTGTTATTATCCGAGA  
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG  
5 GAGTACTTTTTTTAATGTATTTCTTCCTTGGACGAGCAA  
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA  
TTTAAATTTAAAATAATTCAAAATATATCATTTCTTTTA  
TTAGGAATATAAAAATTTTA 3'

10 SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomyces ludwigii*.

5' CCTTCTCAAAGAGTTTTTTTATTCTTTTGGTTGTGAGTG  
ATACTCTTTCCTTTTACAGGGAAGGGGTAACTTGAAAT  
TGTTGCCTAGCAAAGAAGAATTTTGATTGAAATTTCTTG  
15 TTTATTACTATTAGGTTTATCCCAACTAGTGATTATTGA  
GAGTTTTTATTACAGAGTCTTTTCACTTGCTATAATACT  
ATTCTATAAGT 3'

SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
20 AGCAAGGATAAAAATACATTAAATTTATTAGTTTAGTC  
AAGAAAGAATTTTA 3'

SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.

5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT  
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA  
25 TGATTAAAGGTTTCGGCCGTTCAATTATTTTTTTCTTCTCC  
CCCAGTTATCAAGT 3'

SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA  
30 AGTAAGGATAAAAATACATTAAATTTATTAGTTTTAGT  
CAAGAAAGAATTTTA 3'

SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.

5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT  
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA  
TGATTTAAGGTTTCGGCCGTTCAATTATTTTTCTTCTCCC  
5 CCAGTTATCAAGT 3'

SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.

5' CAGAGTTCATGCCCCGAAAGGGTAGACCTCCCACCCTTG  
TGTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG  
CCTTGATGCTCGCCAGAGAATACCAAACTCTTTTAT  
10 TAATGTCGTCTGAGTACTATATAATAGTTA 3'

SEQ ID NO: 22 DNA sequence for the ITS2 of *Botrytis cinerea*.

5' CAACCCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGT  
AATGGCAGGCTCTAAAATCAGTGGCGGCGCCGCTGGGT  
CCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTG  
15 TGCTTCTGCCAAAACCCAAATTTTTCTATGG 3'

SEQ ID NO: 23 DNA sequence for the ITS1 of *Penicillium crustosum*.

5' CCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG  
TTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTA  
GGCCGCCGGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC  
20 CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT  
GAGTGAAAATATAAATTATTA 3'

SEQ ID NO: 24 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCC  
CGATCTCCGGGGGACGGGCCCCGAAAGGCAGCGGCGGCA  
25 CCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCG  
CTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAA  
TTTTTATCCAGG 3'

SEQ ID NO: 25 DNA sequence for the ITS1 of *Penicillium expansum*.

5' CCGAGTGAGGGCCCTTTGGGTCCAACCTCCCACCCGTGT  
 TTATTTACCTCGTTGCTTCGGCGGGCCCGCCTTAAGTGG  
 CCGCCGGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG  
 AAGACACCCCCGAAGTCTGCCTGAAGATTGTCGTCTGA  
 GTGAAAATATAAATTATTTA 3'

SEQ ID NO: 26 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCTC  
 CGATTCCGGGGGACGGGCCCCGAAAGGCAGCGGCGGCAC  
 CGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGC  
 TCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATT  
 TTTATCCAGG 3'

SEQ ID NO: 27 DNA sequence for the ITS1 of *Hanseniaspora guilliermondii*.

5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT  
 CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT  
 TAATTGCGCTGCTTTTTTTAAAGTGTCGCAGTAGAAGTAA  
 TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG  
 TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA  
 AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT  
 TATTATAATTTTTTAACTAAACCAAATTCCTAACGGA  
 AATTTTAAAATAATTTA 3'

SEQ ID NO: 28 DNA sequence for the ITS2 of *Hanseniaspora guilliermondii*.

5' CCTTCTCAAAGATAATTTTTTATTTTTTGGTTGTGGGC  
 GATACTCAGGGTTAGCTTGAAATTGAAGATTGTTTCAAT  
 CTTTTTTAATTCAACACTTAGCTTCTTTGGAGACGCTGT  
 TCTCGCTGTGATGATTTATGAATTTATTCGTTTTACTTT  
 ACAAGGGAAATGGTAATGTACCTTAGGCAAAGGGTTGC  
 TTTTAATATTCATCAAGT 3'

30 SEQ ID NO: 29 DNA sequence for the ITS1 of *Debaryomyces carsonii*.

5' CAGTATTCTTTTTGCCAGCGCTTAAGTGCAGCGGCGAAAT  
 AAACCTTACACACAATGTTTTTTGTTATTACAGGAACTT

TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG  
GTTTAAACTAACTTCAATTTATTTGAACTATTTTCTTAT  
TGAAATGTCACCTTTGTTGATTAAATTCAAAAAATCTTCA  
3'

5 SEQ ID NO: 30 DNA sequence for the ITS2 of *Debaryomyces carsonii*.

5' CTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATACTCT  
TAGTCGAACTAGGCGTTTGCTTGAAATGTATCGGCATGA  
GTGGTACTAGATTAGTGCTTCAGATTTTCAATGTATTA  
GGTTTATCCAACCTCGTTGAATAGTCTGATGGCAAGTGTT  
10 TAGTAACTATGGCTCGGCCTAACAACAACAACAAGT  
3'

SEQ ID NO: 31 DNA sequence for the ITS1 of *Pichia anomala*.

5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA  
CCTTACACACATTGTCTAGTTTTTTTTGAACTTTGCTTTGG  
15 GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA  
TTTTTTTAATGTTAAAACCTTTAACCAATAGTCATGAAA  
ATTTTAAACAAAAATTAAAATCTTCA 3'

SEQ ID NO: 32 DNA sequence for the ITS2 of *Pichia anomala*.

5' CTCTCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCT  
20 GTCAAGGGTTAACTTGAAATATTGACTTAGCAAGAGTG  
TACTAATAAGCAGTCTTTCTGAAATAATGTATTAGGTTC  
TTCCAACCTCGTTATATCAGCTAGGCAGGTTTAGAAGTAT  
TTTAGGCTCGGCTTAACAACAATAAACTAAAAGT 3'

SEQ ID NO: 33 DNA sequence for the ITS1 of *Pichia kluyveri*.

25 5' CTGTGATTTATATCTTATACACATGCGTGAGCGCACCAA  
ACACCTAAAATTGTAATAATACCAGTCACTAAGTTTTAA  
CAAAACA 3'

SEQ ID NO: 34 DNA sequence for the ITS2 of *Pichia kluyveri*.

5' CCTTCTTGCGCAAGCAGAGTTGAGAACAGGCTATGCCTT  
 TTTCGAAATGGAACGTCGTGGACGAAGTGAAGTAACT  
 TTTAGCACGCTTTGGCCGCCGAACTTTAACTAAGC 3'

SEQ ID NO: 35 DNA sequence for the ITS1 of *Candida krusei*.

5 5' CTGTGATTTACTACTACACTGCGTGAGCGGAACGAAAA  
 CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA  
 CAAGAGAAATCTACGAAAAAACAACA 3'

SEQ ID NO: 36 DNA sequence for the ITS2 of *Candida krusei*.

10 5' CCATCTTGCGCGTGCGCAGAGTTGGGGGAGCGGAGCGG  
 ACGACGTGTAAAGAGCGTCGGAGCTGCGACTCGCCTGA  
 AAGGGAGCGAAGCTGGCCGAGCGAACTAGACTTTTTTT  
 CAGGGACGCTTGGCGGCCGAGAGCGAGTGTTGCGAGAC  
 AACAAAAAGC 3'

SEQ ID NO: 37 Oligonucleotide Sequence SXUITS1a.

15 5' TTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAG  
 ACAAGAGATGGAGAGTCCACCC 3'

SEQ ID NO: 38 Oligonucleotide Sequence SXLITS2a.

5' CCGTACTTGCATTATACCTCAAGCACGCAGAGAAACCT  
 CTCTTTGGAAAAAAAAAACA 3'

20 SEQ ID NO: 39 Oligonucleotide Sequence SXUITS1b.

5' TGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACA  
 AGAGATGGAGAGTCCACCCGGG 3'

SEQ ID NO: 40 Oligonucleotide Sequence SXLITS2b.

25 5' CGACCGTACTTGCATTATACCTCAAGCACGCAGAGAAA  
 CCTCTCTTTGGAAAAAAAAAAC 3'

SEQ ID NO: 41 Oligonucleotide Sequence SLUITS1a.

5' CTGTTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGG  
TTGTTGATTGCTTTGGCCTGCGCTTAAGTGCAGCGGCTAG  
TAGTTGATGATTTTGTATTATCCGAGACGAA 3'

5 SEQ ID NO: 42 Oligonucleotide Sequence SLLITS2a.

5' TAATAAAAAGTCTCAATAATCACTAGTTGGGATAAACC  
TAATAGTAATAACAAGAAATT 3'

SEQ ID NO: 43 Oligonucleotide Sequence SLUITS1b.

10 5' TTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGGTTG  
TTGATTGCTTTGGCCTG 3'

SEQ ID NO: 44 Oligonucleotide Sequence SLLITS2b.

5' CTGTAATAAAAAGTCTCAATAATCACTAGTTGGGATAA  
ACCTAATAGTAATAACAAGAAAT 3'

SEQ ID NO: 45 Oligonucleotide Sequence BRUITS1a

15 5' GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG  
CAAGCCCGTGCAGACACGTGGATAAGCAAGGATAAAAA  
TACAT 3'

SEQ ID NO: 46 Oligonucleotide Sequence BRLITS2a.

20 5' ACTGGGGGAGAAGAAAAAATAATGAACGGCCGAAAC  
CTTAAATCATTATCCCCTCACTCTCCCCTCTTTCCTTTGA  
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3'

SEQ ID NO: 47 Oligonucleotide Sequence BRUITS1b.

5' GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG  
CAAGCCCGTGCAGACACG 3'

SEQ ID NO: 48 Oligonucleotide Sequence BRLITS2b.

5' AACTGGGGGAGAAGAAAAATAATGAACGGCCGAAAC  
CTTAAATCATTATCCCCTCACTCTCCCCTCTTTCCTTTGA  
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3'

5 SEQ ID NO: 49 Oligonucleotide Sequence BCUITS1a.

5' TGGCGAGCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAA  
TACCAAACTCTTTTAT 3'

SEQ ID NO: 50 Oligonucleotide Sequence BCLITS2.

10 5' GCGCCGCCACTGATTTTAGAGCCTGCCATTACTGACATA  
GACTCAATACCAAGCTAA 3'

SEQ ID NO: 51 Oligonucleotide Sequence BCUITS1b.

5' GCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAATACCAA  
AACTCTTTTATTAATGT 3'

SEQ ID NO: 52 Oligonucleotide Sequence PXUITS1a.

15 5' CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTACCT  
TGTTGCTTCGGCGGGCCCGCC 3'

SEQ ID NO: 53 Oligonucleotide Sequence PXLITS2a.

5' TCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAA  
AGCCCCATACGCTCGAGGACCGGACGCGGTGC 3'

20 SEQ ID NO: 54 Oligonucleotide Sequence PXUITS1b.

5' CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTACCTC  
GTTGCTTCGGCGGGCCCGC 3'

SEQ ID NO: 55 Oligonucleotide Sequence PXLITS2b.

5' AGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCC  
ATACGCTCGAGGACCGG 3'

SEQ ID NO: 56 Oligonucleotide Sequence HGUITS1.

5 5' AATCTTGCTTGAATCTCAGTCAACGTTTACACACATTGG  
AGTTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCG  
AAAGGTTCAAGGCAAAAAAC 3'

SEQ ID NO: 57 Oligonucleotide Sequence HGLITS2.

10 5' TAAATACATCACAGCGAGAACAGCGTCTCCAAAGAAGC  
TAAGTGTTGAATTAAAAAAGATT 3'

SEQ ID NO: 58 Oligonucleotide Sequence DXLITS2a.

5' CTCATGCCGATACATTTCAAGCAAACGCCTAGTTCGACT  
AAGAGTATCACTCAATACCAA 3'

SEQ ID NO: 59 Oligonucleotide Sequence DXLITS2b.

15 5' TAAACACTTGCCATCAGACTATTCAACGAGTTGGATAA  
ACCTAATACATTGAAAAATCTGA 3'

SEQ ID NO: 60 Oligonucleotide Sequence PAUITS1.

5' TTGCTTTGGGTGGTGAGCCTGGCTTACTGCCCAAAGGTC  
TAAACACATTTTTTTAATGT 3'

20 SEQ ID NO: 61 Oligonucleotide Sequence PALITS2.

5' ATACATTATTTTCAGAAAGACTGCTTATTAGTACACTCTT  
GCTAAGTCAATATTTCAAGTTA 3'

SEQ ID NO: 62 Oligonucleotide Sequence PKLITS2.

5' GCGGCCAAAGCGTGCTAAAAGTTTAGTTCACTTCGTCCA  
CGACGTTCCATTTCGAAAAAGG 3'

SEQ ID NO: 63 Oligonucleotide Sequence CKUITS1.

5 GCGGAACGAAAACAACAACACCTAAAATGTGGAATATA  
GCATATAGTCGACAAGAGAAATC 3'

SEQ ID NO: 64 Oligonucleotide Sequence CKLITS2.

5' TCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTC  
CGCTCCGCTCCCCCAACTCT 3'

10 SEQ ID NO: 65 Oligonucleotide Sequence SXU99.

5' CTTTACTGGGCAAGAAGAC 3'

SEQ ID NO: 66 Oligonucleotide Sequence SXL658.

5' AAGCACGCAGAGAAACC 3'

SEQ ID NO: 67 Oligonucleotide Sequence SXU102.

15 5' TTACTGGGCAAGAAGACAAG 3'

SEQ ID NO: 68 Oligonucleotide Sequence SXL661.

5' CTCAAGCACGCAGAGAA 3'

SEQ ID NO: 69 Oligonucleotide Sequence SLU85.

5' GACTTTTCTTGGGGAGAG 3'

SEQ ID NO: 70 Oligonucleotide Sequence SLL635.

5' TCACTAGTTGGGATAAACCT 3'

SEQ ID NO: 71 Oligonucleotide Sequence SLU88.

5' TTTTCTTGGGGAGAGG 3'

5 SEQ ID NO: 72 Oligonucleotide Sequence SLL636.

5' ATCACTAGTTGGGATAAACCT 3'

SEQ ID NO: 73 Oligonucleotide Sequence SLU136.

5' CGGCTAGTAGTTGATGATT 3'

SEQ ID NO: 74 Oligonucleotide Sequence SLL634.

10 5' CACTAGTTGGGATAAACCTA 3'

SEQ ID NO: 75 Oligonucleotide Sequence BRU53A.

5' ATTACAGGATGCTGGGC 3'

SEQ ID NO: 76 Oligonucleotide Sequence BRU53B.

5' ATTACAGGATGCTGGG 3'

15 SEQ ID NO: 77 Oligonucleotide Sequence BRU77.

5' CGTGCAGACACGTGGAT 3'

SEQ ID NO: 78 Oligonucleotide Sequence BRL339.

5' CTTTGAAGAAAACACCCTC 3'

SEQ ID NO: 79 Oligonucleotide Sequence BRL367.

5' AATCATTATCCCCTCACTC 3'

SEQ ID NO: 80 Oligonucleotide Sequence BRL390.

5' TAATGAACGGCCGAAAC 3'

5 SEQ ID NO: 81 Oligonucleotide Sequence BCU136.

5' TTGTATGCTCGCCAGAG 3'

SEQ ID NO: 82 Oligonucleotide Sequence BCL393.

5' GCCTGCCATTACTGACA 3'

SEQ ID NO: 83 Oligonucleotide Sequence BCU142.

10 5' GCTCGCCAGAGAATACC 3'

SEQ ID NO: 84 Oligonucleotide Sequence PXU87.

5' ACCCGTCTTTATTTTACCTT 3'

SEQ ID NO: 85 Oligonucleotide Sequence PXL495.

5' CTACAGAGCGGGTGACA 3'

15 SEQ ID NO: 86 Oligonucleotide Sequence PXU86.

5' CACCCGTGTTTATTTTACCT 3'

SEQ ID NO: 87 Oligonucleotide Sequence PXL482.

5' CAAAGCCCCATACGC 3'

SEQ ID NO: 88 Oligonucleotide Sequence PXL491.

5' AGCGGGTGACAAAGC 3'

SEQ ID NO: 89 Oligonucleotide Sequence HGU193.

5' CAACGTTTACACACATTGG 3'

5 SEQ ID NO: 90 Oligonucleotide Sequence HGU231.

5' AATTCTTTCTGCTTTGAATCG 3'

SEQ ID NO: 91 Oligonucleotide Sequence HGL601.

5' CAGCGTCTCCAAAGAAGCTAA 3'

SEQ ID NO: 92 Oligonucleotide Sequence DXL447.

10 5' GCAAACGCCTAGTTCGACTAA 3'

SEQ ID NO: 93 Oligonucleotide Sequence DXL526.

5' ATTCAACGAGTTGGATAAACC 3'

SEQ ID NO: 94 Oligonucleotide Sequence PAU133.

5' GGCTTACTGCCCAAAGGTC 3'

15 SEQ ID NO: 95 Oligonucleotide Sequence PAL451.

5' TGCTTATTAGTACACTCTTGC 3'

SEQ ID NO: 96 Oligonucleotide Sequence PKL356.

5' GTTTAGTTCACTTCGTCCACG 3'

SEQ ID NO: 97 Oligonucleotide Sequence CKU104.

5' CCTAAAATGTGGAATATAGCA 3'

SEQ ID NO: 98 Oligonucleotide Sequence CKL354.

5' ACGCTCTTTACACGTCGTC 3'

5 SEQ ID NO: 99 Oligonucleotide Sequence ITS5.

5' GGAAGTAAAAGTCGTAACAAGG 3'

SEQ ID NO: 100 Oligonucleotide Sequence ITS4.

5' TCCTCCGCTTATTGATATGC 3'

We claim:

1. An isolated double stranded nucleic acid selected from the group consisting of SEQ ID NOS: 13 to 36.
2. An isolated nucleic acid which specifically hybridizes with the  
5 nucleic acid of claim 1.
3. An oligonucleotide sequence for identification of a fermentation-related microorganism, wherein said sequence is selected from the group consisting of SEQ ID NOS: 37 to 64.
4. An oligonucleotide primer which is a fragment of the sequences  
10 according to claim 3, and which specifically hybridizes to the ITS1 or ITS2 of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri* or *Candida krusei*.
- 15 5. An oligonucleotide primer for identification of a fermentation-related microorganism, wherein said primer is selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide  
20 bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

6. A pair of oligonucleotide primers for use in the amplification-based detection of an internal transcribed spacer sequence of a fermentation-related microorganism, wherein the primers are selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

7. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 65 and SEQ ID NO: 66.

8. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 67 and SEQ ID NO: 68.

9. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 69 and SEQ ID NO: 70.

10. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 71 and SEQ ID NO: 72.

11. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 73 and SEQ ID NO: 74.

12. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 75 and SEQ ID NO: 78.

13. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 76 and SEQ ID NO: 79.

14. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 77 and SEQ ID NO: 80.

5 15. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 81 and SEQ ID NO: 82.

16. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 85 and SEQ ID NO: 83.

10 17. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 84 and SEQ ID NO: 85.

18. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 87.

19. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 88.

15 20. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 89 and SEQ ID NO: 91.

21. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 90 and SEQ ID NO: 91.

20 22. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 94 and SEQ ID NO: 95.

23. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 97 and SEQ ID NO: 98.

24. A method for detection of a fermentation-related microorganism comprising:

- 5 (a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage;
- 10 (b) amplifying a part of the internal transcribed spacer sequence of said fermentation-related microorganism using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 6; and
- (c) visualizing said amplified part of the internal transcribed spacer sequence to determine whether said fermentation-related microorganism is present.

15 25. The method according to claim 24, wherein said fermentation-related microorganism is selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*,  
20 *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*.

26. The method according to claim 24, wherein said fermentation culture or fermentation beverage is a wine fermentation culture or wine fermentation beverage.

27. The method according to claim 24, wherein the pair of  
25 oligonucleotide primers comprises SEQ ID NO: 65 and SEQ ID NO: 66.

28. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 67 and SEQ ID NO: 68.

29. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 69 and SEQ ID NO: 70.

5 30. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 71 and SEQ ID NO: 72.

31. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 73 and SEQ ID NO: 74.

10 32. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 75 and SEQ ID NO: 78.

33. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 76 and SEQ ID NO: 79.

34. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 77 and SEQ ID NO: 80.

15 35. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 81 and SEQ ID NO: 82.

36. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 82 and SEQ ID NO: 83.

20 37. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 84 and SEQ ID NO: 85.

38. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 87.

39. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 88.

5        40. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 89 and SEQ ID NO: 91.

41. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 90 and SEQ ID NO: 91.

10       42. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 94 and SEQ ID NO: 95.

43. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 97 and SEQ ID NO: 98.

15       44. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising an oligonucleotide primer according to claim 5.

45. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising a pair of oligonucleotide primers according to claim 6.