1	AN INNOVATIVE OLIGONUCLEOTIDE MICROARRAY TO DETECT SPOILAGE
2	MICROORGANISMS IN WINE
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### 27 Abstract

28 The aim of this investigation has been the design and validation of an oligonucleotide microarray in 29 order to detect 17 different wine-spoilage microorganisms, i.e. 9 yeasts, 5 lactic bacteria and 3 30 acetic acid bacteria species. Furthermore, several strains belonging to these species has been found 31 to produce undesirable compounds for wine consumers. Oligonucleotide probes specific for each 32 microorganism were designed to target the intergenic spacer regions (ISR) between 18S-5.8S region for yeasts and 16S-ITS1 region for bacteria. Prior to hybridization the ISR were amplified by 33 34 combining reverse transcriptase and polymerase chain reactions using a designed consensus primer. 35 Each oligonucleotide-probes exclusively recognized its target without undesired aspecific cross-36 hybridizations. Under our experimental condition, the microarray assay analysis was able to detect 37 the amount of DNA equivalent to 24 (Saccharomyces cerevisiae), 160 (Lactobacillus brevis) and 38 124 (Gluconobacter oxydans) cells, three species chosen as experimental models for the three studied microbial classes. Moreover, a novel procedure that allowed the extraction of genomic 39 40 DNA from a mixture of eukaryotic and prokaryotic cells from contaminated wine was developed. 41 The obtained results confirm that the microarray assay is able to detect specifically different 42 spoilage microorganisms present in mixture in contaminated wines. For the first time the microarray 43 methodology has been applied for the simultaneous identification of different mixed population of spoilage yeast and bacteria directly isolated from wine, thus indicating the practicability of 44 45 oligonucleotide microarrays as a contamination control in wine industry.

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- 47 Keywords: biotechnology; wine; wine spoilage; microarray; PCR.
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## 50 **1. Introduction**

51 Yeasts and bacteria play important roles in winemaking such as catalysing the rapid, complete and 52 efficient conversion of grape sugar into ethanol as well as reducing wine acidity, improving 53 microbiological stability and enhancing wine aroma and flavour. However, under uncontrolled 54 conditions, microbial activity may also be disadvantageous for wine quality (Bartowsky, 2009; M. 55 Du Toit & Pretorius, 2000). Wine deterioration due to spoilage microorganisms is becoming a major problem for wine industry because can cause significant economic losses (Krisch, 56 57 Chandrasekaran, Kadaikunnan, Alharbi, & Vágvölgyi, 2016; Luo, Schmid, Grbin, & Jiranek, 2012) 58 also in the light of wine production increased scale all over the world (Mariani, Pomarici, & Boatto, 2012). Moreover, wine consumers, nowadays, demand milder processing, preservation and storage 59 60 conditions that also contribute to increase wine spoilage drawback (Lockshin & Corsi, 2012). 61 Microbial spoilage can occur at different stages during wine production or storage (Rankine, 1995; 62 Tristezza et al., 2010). Many lactic acid bacteria genera, such as Lactobacillus and Pediococcus, are 63 among the most concerning microbial contaminants and are well known for their capacity to 64 depreciate wine (Bartowsky, 2009) as well as to produce undesirable compounds for wine 65 consumers health such as biogenic ammines (Mateo, Torija, Mas, & Bartowsky, 2014; Russo et al., 66 2016). Also wine alterations due to activity and growth of contaminant yeasts in processed and 67 bottled wines is a serious concern for wine industry (Krisch et al., 2016; Loureiro & Malfeito-68 Ferreira, 2003); wine spoilage yeasts belong to several genera including Dekkera/Brettanomyces, 69 Hanseniaspora, Candida, Pichia, Zygosaccharomyces (Enrique et al., 2007; Loureiro & Malfeito-70 Ferreira, 2003). Furthermore, some strains belonging to these species were able to synthesize 71 histamine and cadaverine during must fermentation (Tristezza et al., 2013). Even the species 72 Saccharomyces cerevisiae might be considered as a spoilage organism when associated with re-73 fermentation of bottled wines (Deak, 2007; Loureiro & Malfeito-Ferreira, 2003; Tristezza et al., 74 2010).

75 Consequently, to prevent economical losses, it would be helpful to have tools able to 76 simultaneously identify the undesirable microorganisms. Microarrays approach has been applied for 77 microbial identification and detection in food stuffs (McLoughlin, 2011; Rasooly & Herold, 2008).

78 Microarray technology based on species-specific sequences is rapid, sensitive and unambiguously 79 allows identification of single species (Southern, 2001) into a mixed microbial community. For 80 instance, the sensitive and specific detection and identification of ascomycetes has been carried out 81 drawing primer pairs complementary to the highly conserved 18S and 5.8S regions of rRNA genes 82 and using oligonucleotide capture probes complementary to the more variable ITS1 regions present 83 in multiple copies in fungal and yeast genomes, that allow a discrimination of fungal and yeast 84 species (Healy et al., 2004; Hsiao et al., 2005; Spiess et al., 2007). As far as bacterial detection is concerned, bacterial 16S rRNA genes, including nine "hyper-variable regions" (V1-V9), 85 86 characterized by significant sequence diversity among different bacterial genera, have been utilized 87 for species identification (Huws, Edwards, Kim, & Scollan, 2007).

88 Indeed, microarray applications could play an important role for safety and quality supervision, 89 particularly in the food and beverage industries. DNA microarray tests have been developed for 90 identification of food-borne bacterial pathogens in the environment (Call, Borucki, & Loge, 2003), 91 in different food commodities (Wang et al., 2007) and also for the simultaneous detection of 92 numerous pathogenic and non-pathogenic bacteria in raw milk (Giannino et al., 2009). Moreover, 93 Weber and coworkers (2008) developed and applied an oligonucleotide microarray able to detect 94 and identify viable bacterial species, belonging to the genera Lactobacillus, Megasphaera, Pediococcus and Pectinatus, recognized (Priest, 2006) as biological agents of beer spoilage. In 95 96 general extensive studies have been carried out to optimize efficient molecular methods for the 97 detection of wine spoilage microorganisms (Ivey & Phister, 2011), but none of them can ensure the 98 simultaneous detection of numerous eukaryotic and prokaryotic undesired microorganisms.

99 The aim of the present study was to develop an alternative diagnostic method for the rapid and 100 simultaneous detection of wine spoilage yeasts and bacteria directly extracted from contaminated 101 wines. A prototype oligonucleotide microarray, based on species-specific probes targeting rDNA-102 specific regions, was designed and assessed as able to detect 17 different wine-spoilage 103 microorganisms, i.e. 9 yeasts, 5 lactic bacteria and 3 acetic acid bacteria species. To the best of our 104 knowledge, this is the first report concerning a single microarray-based assay for the concurrent 105 identification of different eukaryotic and prokaryotic microorganisms responsible for wine spoilage.

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## 108 **2. Materials and methods**

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## 110 2.1 Microbial strain cultures and DNA extraction

111 Yeast and bacterial strains used in this study (Table 1) were store at -80 °C in 50% glycerol. 112 Diagnostic ability of the DNA microarray to detect microorganisms was determined using genomic 113 DNAs extracted from test strains in laboratory media: YPD (1% yeast extract, 2% peptone, 2% 114 glucose) for yeasts, MRS (Oxoid, Basingstoke, UK) for lactic acid bacteria and GY (5% glucose, 115 1% yeast extract) for acetic acid bacteria. Genomic DNAs from pure yeast and bacterial cultures 116 were extracted using the methods respectively described by Tristezza et al. (2009) and Cappello et 117 al. (2008). The concentration of the extracted DNA was measured using a NanoDrop 118 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

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# 120 2.2 DNA extraction from yeast/bacterial mixed cultures isolated from artificially infected wine

Genomic DNAs of mixed bacterial/yeast cells were directly extracted from artificially infected wine. The wine used was first micro filtrate and subsequently artificially contaminated with known amounts of microorganisms. The contaminated wines were centrifuged and the sediment was suspended in a suitably formulated suspension buffer. Briefly, one millilitre of artificially contaminated wine was centrifuged for 5 minutes at 8000 ×g and thereby the wine was removed. The pellet obtained was washed with 1 mL of Buffer A (60 mM Tris-HCl pH 7.4, 10 mM EDTA 127 pH 7.4), centrifuged for 5 minutes at 8000 ×g and the supernatant was discarded. The washed pellet 128 was re-suspended using 8 mg of lysozyme (Sigma-Aldrich, Milan, Italy) + 0.8 mg lyticase (Sigma-129 Aldrich, Milan, Italy), in a final volume of 200 µL of Buffer A The slurry was mixed by vortex and 130 incubated at 37°C for 1 hour. Then 400 µg of RNase (20 µL; Sigma-Aldrich, Milan, Italy) were 131 added to the mixture and incubated for 2 minutes at 25°C. After a further addition of 400 µg of 132 Proteinase K (20 µL) and 200 µL of Lysis solution [10 mM Tris (pH 8.0), 10 mM EDTA, and 2.0% 133 SDS], the mixture was mixed by vortex and incubated at 55°C for 10 minutes. The lysate was added 134 with 200 µL of absolute ethanol and the genomic DNAs were afterward extracted using the 135 GenElute<sup>™</sup> Bacterial Genomic DNA Kit (Sigma-Aldrich, Milan, Italy) according to the 136 manufacturer's instructions.

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### 138 2.3 Primers and probes design

Primers and oligonucleotide probes used for identification of microorganisms, were designed using 139 140 the reference sequences (18S-5.8S rRNA genes region for yeasts and 16S rRNA Gene-ITS1 141 [Internal Transcribed Spacer] region for bacteria) available in the GenBank database of the NCBI 142 homepage (http://www.ncbi.nlm.nih.gov/). The selected sequences were compared with at least one 143 sequence of the same species in the database and they were aligned with ClustalX implemented in 144 BioEdit 7.0.5.2 software (Hall, 1999) for the selection of regions suitable for oligonucleotide probes 145 design. The oligonucleotide probes were designed using Primer 3.0 program (http://www-146 genome.wi.mit.edu/genome\_software/other/primer3.html) and the following parameters were 147 applied: GC-content between 35 and 60%, maximum Tm set at 58°C and probe length between 20 148 and 30 bp. Probe sequences were tested for duplex and hairpin formation with the Oligo Analyzer 149 3.1 (http://www.idtdna.com) software. Each designed probe sequence was optimised by deleting or 150 adding bases at both ends, according to melting temperature and duplex formation. Oligonucleotide 151 probes were checked by BLAST analysis (http://www.ncbi. nml.nih.gov/BLAST/) against sequences from all available species within the database. 152

Oligonucleotide probes (Invitrogen) were synthesized adding at the 5' end 12 carbon residues as
spacer and a 5' NH<sub>2</sub> group.

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### 156 2.4 Construction of DNA-microarrays

The oligonucleotide probes were modified by adding a sequence of 12 carbon atoms, linked to an amino group, at 5' end. By this organic spacer the oligonucleotide probe is spaced out the slide surface and fully exposed and available to bind target DNA. Interaction between the slide and the oligonucleotide probes takes place by a covalent bond between the amino group of the oligonucleotide and the epoxide coating the slide surface. The oligonucleotide probes were deposited in duplicate on the epoxy slide either manually, according to the scheme reported in Figure 1, or automatically, according to the scheme reported in Figure 4.

164 Probes were suspended in 2X saline-sodium citrate (SSC) buffer (1X SSC = 0.15 M sodium 165 chloride, 15mM trisodium citrate, pH 7) at a final concentration of 40 µM and distributed in a 96-166 well plate. The oligonucleotide probes were spotted on the epoxy-coated glass slides (Nexterion® 167 Slide E) by contact printing using a robotic spotting SpotArrayTM 24 (Perkin Elmer) by the 168 following protocol: 55-60% humidity; pin contact time of 400 msec; deposition volume of 10 nL; 169 spot size diameter of 100 µm; distance between two spots of 400 µm. The improvement of 170 background and sensitivity of the spot fluorescence signals was achieved by preliminary study using a manual contact printing MicroCaster<sup>TM</sup> Arrayer (Whatman). This method allows a 171 deposition volume of 50-70 nL; spot size diameter of 400-700 µm; distance between two spots of 172 173 900-1300 µm. The variability of the spot size is due to a different pin contact time, performed by a 174 manual printing in order to allow the covalent bond between the epoxide group on the slide surface 175 and the amino group at 5' end of oligonucleotide probes. After deposition, the slide was incubated 176 in a humid chamber at room temperature for 2 hours and then stored at room temperature.

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178 2.5 DNA labelling

179 The target DNA was labelled using one of the two primer, forward or reverse, labelled at the 5' end 180 with the Cyanine 5 (Cy5) fluorochrome (Invitrogen<sup>™</sup> Life Technologies, USA) by a Linear-After-181 The-Exponential-PCR (LATE-PCR). The LATE-PCR is an asymmetric PCR based on the 182 amplification of a single strand of Cy5-labelled DNA at higher amount compared to the 183 complementary strand, with predictable kinetics for many cycles beyond the exponential phase 184 (Rice et al., 2007). LATE-PCR increases the number of strands labelled with cyanine in order to 185 reduce the unlabelled complementary strands that, during the hybridization step on microarray, for 186 competition effect is able to limit the binding with oligonucleotide probes immobilized on the array. 187 The LATE-PCR method is a composed by two sequential steps that were carried out as following.

188 Traditional Exponential-PCR. The base master mix consisted of 5 µL reaction buffer [10X, 189 Euroclone; 160 mM (NH<sub>4</sub>)<sub>2</sub>S0<sub>4</sub>, 670 mM TRIS HCl pH 8.8; 0.1% Tween-20], 3 mM MgCl<sub>2</sub> 50 (, 190 0.2 mM,dNTP mix (Invitrogen, USA), 0.2 µM of each genus primer (Cy5-primer and reverse or 191 forward prime), 2 µL of DNA template, 2.5 units Taq polymerase (, Euroclone, Italy) and sterile 192 water to 50 µl. Following an initial denaturation at 95°C for 4 min, products were amplified by 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and elongation at 72°C for 45 s. 193 194 Amplification was followed by a final extension at 72°C for 5 min. 10 µL of product (1/5 of PCR 195 reaction volume) was used for the subsequent Linear-PCR.

196 Linear-PCR. Five µL of reaction buffer [10X, Euroclone; 160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 670 mM 197 TRIS HCl pH 8.8; 0.1% Tween-20], 3 mM MgCl<sub>2</sub> 50 mM, 0.2 mMdNTP mix (10, Invitrogen, USA), 0.2µM of Cy5-primer, 10 µL of the previously obtained PCR product (1/5 of Exponential-198 199 PCR reaction volume), 2.5 µL units Taq polymerase (Euroclone, Italy) and sterile water to 50 µL. 200 Following an initial denaturation at 95°C for 2 min, products were amplified by 15 cycles of 201 denaturation at 95°C for 20 s, annealing at 58°C for 20 s and elongation at 72°C for 20 s. 202 Amplification was followed by a final extension at 72°C for 1 min. The amplified Cy5-labelled 203 DNA was purified by illustra MicroSpin G-50 Columns (GE Healthcare, USA) and diluted (1:2 v/v) 204 with hybridization buffer for microarray analysis.

# 206 2.6 Microarray hybridization

Before hybridizations, the spotted slide was incubated twice for 2 min in a solution of 1 mM HCl, then 10 min in a solution of 100 mM KCl, washed twice in sterile water and blocked for 15 min at  $50^{\circ}$ C with Blocking solution [50mM ethanolamine; 0.1% SDS, 0.1M Tris, pH 9], in order to inactivate residual reactive epoxy groups. After two washing steps with sterile water, the slide was dried by centrifugation for 5 min at 200 ×g and placed into the hybridization chamber.

212 The Cy5-labelled DNA diluted (1:2 v/v) with hybridization buffer (3X SCC; 0,1% SDS; 30% 213 deionised form amide, Sigma), was denatured at 95°C for 3 min and then immediately applied into 214 the well of the hybridization chamber (Nexterion® IC-16, Schott, Germany). Wells were covered 215 with a plastic layer to avoid evaporation during hybridization and incubated for 4 hours (or 216 overnight) at 42°C. After hybridization, the slide was removed from hybridization chamber and 217 washed in 4X SSC for 1 min, twice in 2X SSC with 0.1% SDS for 5 min, in 0.2X SSC for 1 min 218 and finally in 0.1X SSC for 1 min. After the washing steps, the microarray was dried by 219 centrifugation for 4 min at  $200 \times g$  and analyzed at the laser scanner.

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### 221 2.7 Scanning and data analysis

The fluorescence signal for Cy5 was determined at 633 nm by using a ScanArray Express laser scanner (Perkin-Elmer, Foster City, CA, USA). Slides were scanned with a resolution of 10 µm and at the same laser power and sensitivity level of the photomultiplier. The draw fluorescence data acquired were stored as image files in TIFF format and analyzed quantitatively by ScanArray Express software (Perkin-Elmer, USA). The fluorescence signal of each spot was calculated as the difference between the mean of pixel intensities and the mean of background fluorescence signals, defined by surrounding pixel intensity according to Heiskanen et al. (2000).

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### 230 **3. Results**

## 232 3.1 Bioinformatic analysis and design of oligonucleotide probes for microarray construction

233 The bioinformatic analysis of rDNA cistron sequences (18S-5.8S rRNA genes region for yeasts and 234 16S rRNA gene-ITS1 region for bacteria) belonging to different strains of each of the 17 species 235 (Table 2) has produced three separate multiple alignments, deriving respectively from yeast, lactic 236 and acetic bacteria rDNA sequences. Each output file allowed to highlight both conserved regions 237 (on which the primer pair used for the preparation of the target DNA has been built) and non-238 common regions (on which the oligonucleotide probes to be immobilized on the microarray slides 239 have been constructed). For yeasts, the forward primer has been identified on the 18S region and the 240 reverse primer on the 5.8S region, whereas for bacteria the forward primer has been identified on 241 the 16S region and the reverse primer on the ITS1 region (Table 3). Size of the different specific 242 fragments is indicated in Table S1 and the obtained amplicons are shown in Figure S1. In the case 243 of lactic acid bacteria, a 300 bp long amplicon was obtained. The forward primer was used in the 244 preparation of the each of the three specific-target DNAs by LATE-PCR assay (Table 3). A species-245 specific oligonucleotide probe for each microorganism was designed in the region between the two 246 sequences used to draw the two primers. Each primer was constructed to be 20 nucleotides long and 247 with hybridization temperature (Tm) of 58-60°C (Table 4) and their ability to exclusively 248 recognized its species-specific target was confirmed by separately submitting each primer sequence 249 to BLAST analysis (Figure S2).

Seventeen oligonucleotide probes were designed in order to specifically recognize and hybridize with the target DNA of the corresponding microorganism, in particular 9 oligonucleotide probes for the nine species of yeasts and 8 oligonucleotide probes for the acetic acid and lactic acid bacteria species were constructed, which were immobilized on the epoxy slide.

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### 255 3.2 Labelling of the target DNA and microarray hybridization

256 Preparation of the target DNA was carried out by PCR using, in each amplification, the forward 257 primer labelled with the Cy5 fluorescent tag. In order to obtain a more evident signal, the target 258 DNA synthesis was carried out by using the Linear-After-The-Exponential (LATE)-PCR, which 259 allowed to obtain an increased signal with a lower background noise. The Figure 1 shows the results 260 obtained hybridizing separately the 17 target DNAs with the DNA microarray. In all assays, a very 261 low background noise was obtained. Furthermore, the experimental conditions used produced a high intensity fluorescence signal strictly corresponding to the specific oligonucleotide probe 262 263 immobilized on the epoxy glass slide. This indicates the absence of aspecific cross-hybridization signals. In fact, each of the 17 oligonucleotide probes exclusively recognized its target not 264 265 hybridizing with any target of the other yeast or bacteria species.

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### 267 3.3 Microarray sensitivity assessment

268 To assess sensitivity limit of the microarray, the minimal detectable concentration of target DNA 269 was determined. The sensitivity test was carried out using three model microorganisms, namely 270 Saccharomyces cerevisiae (yeast), Gluconobacter oxydans (acetic acid bacteria) and Lactobacillus 271 brevis (lactic acid bacteria). Different solutions containing decreasing amounts of DNA of the three 272 model microorganisms (i.e. 50 pg, 10 pg, 2 pg and 0.4 pg) were prepared and used as template in 273 LATE-PCR reactions using respectively the primer pairs Liev For Cy5/Liev Rev, 274 Acet\_For\_Cy5/Acet\_Rev, Latt\_For\_Cy5/Latt\_Rev. The electrophoretic analysis of LATE-PCR 275 products indicate that the expected amplicons are visible when 50 and 10 pg of template DNA were 276 used, while no products are observed when using 2 and 0.4 pg of template DNA (Figures S3).

When the LATE-PCR products of the three model microorganisms DNAs (at the four different concentrations) were utilized for hybridization of the microarray slide, the hybridization signal is present in all samples. Moreover, a very low level of background noise and no cross-reactions were observed, thus confirming the high specificity of each target DNA (Figure 2). Under the experimental condition used, the microarray was able to detect target DNA obtained from LATE- PCR performed with 0.4 pg of template, that means the amount of DNA corresponding to 24 (*S. cerevisiae*), 160 (*L. brevis*) and 124 (*G. oxydans*) cells.

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### 285 3.4 Simultaneous detection of microorganisms from DNA mixtures

A further step in the optimization of the microarray was the simultaneous amplification of target DNAs deriving from a mixture of different microorganism in order to verify the specific production of the expected target DNAs and the absence of undesired non-specific amplification products. Thus we developed a procedure for extracting genomic DNA from a mixture of prokaryotic and eukaryotic microbes directly from contaminated wine by the concurrent addition of lysozyme and lyticase enzymes, able to respectively degrade the cellular wall of bacteria and yeasts.

292 Four separate amplification reactions were set up using simultaneously the three pairs of primers 293 Liev For Cy5/Liev Rev (yeasts), Latt For Cy5/Latt Rev (lactic acid bacteria) and 294 Acet\_For\_Cy5/Acet\_Rev (acetic acid bacteria) and, as substrate, the following mixtures of genomic 295 DNAs, at the concentration of 20 pg/µL each: Mix 1, S. cerevisiae and Schizosaccharomyces 296 pombe; Mix 2, S. cerevisiae, Pichia membranifaciens and L. brevis; Mix 3, S. cerevisiae, Candida 297 stellata, L. brevis and G. oxydans; Mix 4, S. cerevisiae, Pichia anomala, P membranifaciens, L. 298 brevis and G. oxydans (Figure S4). The four different target DNA preparations were used to 299 hybridize separately four identical arrays. Figure 3 shows the results obtained after the four 300 independent hybridizations carried out using the above-described four mixture of target DNAs. In 301 all the performed experiments a highly specific fluorescence signal was observed. A very low level 302 of background noise and no undesired cross-hybridization signal were obtained. The results 303 obtained clearly indicate that the microarray is useful to identify specifically the DNA of different 304 microorganisms (yeasts, lactic acid and acetic acid bacteria) present in the mixture and to assess that 305 the contemporary presence of different target DNAs in the hybridization mixture does not cause any 306 interference among the different amplified targets.

### 308 *3.5 Detection of microorganisms from spoiled wine*

In order to detect simultaneously one or more microorganisms directly from spoiled wines, a procedure was set up that allowed the extraction of genomic DNA from a mixture of eukaryotic and prokaryotic cells. The wine used was first micro filtered and then artificially contaminated using a mixture containing known cell concentration of model microorganisms, representative of the three classes of spoilers, *S. cerevisiae* (yeasts), *L. brevis* (lactic acid bacteria) and *A. aceti* (acetic bacteria), mixed in the following proportions:

A) S. cerevisiae:  $10^6$  CFU/mL; L. brevis:  $10^6$  CFU/mL; A. aceti:  $10^6$  CFU/mL.

B) S. cerevisiae: 10<sup>5</sup> CFU/mL; L. brevis: 10<sup>5</sup> CFU/mL; A. aceti: 10<sup>5</sup> CFU/mL

317 C) S. cerevisiae: 10<sup>4</sup> CFU/mL; L. brevis: 10<sup>4</sup> CFU/mL; A. aceti: 10<sup>4</sup> CFU/mL

318 D) S. cerevisiae: 10<sup>3</sup> CFU/mL; L. brevis: 10<sup>3</sup> CFU/mL; A. aceti: 10<sup>3</sup> CFU/mL

319 After incubation in wine, the four microorganisms mixtures were concentrated by centrifugation 320 and each sediment was separately re-suspended in the suspension buffer formulated ad hoc during 321 this work. In particular the optimization of two enzymatic reactions carried out simultaneously was 322 achieved by adding to the aforementioned buffer the optimal amount of lysozyme and lyticase that 323 are respectively able to degrade the cell wall of bacteria and yeasts. Genomic DNA released in the 324 lysate was purified by chromatography on a silica gel column. Reproducible amplification of the 325 expected products was obtained by using as substrate the DNA extracted from all the mixtures 326 except that from mixture D. Target DNAs amplified from the genomic template extracted from 327 Mixture C were used in the hybridization reaction with the microarray (Figure 4). The experimental 328 conditions adopted have produced a high intensity fluorescence signal corresponding to the specific 329 oligonucleotide probe for A. aceti, L. brevis and S. cerevisiae, thus indicating that each DNA target 330 recognizes only its specific oligonucleotide probe without cross-interference and background noise. 331 The above described procedure was validated by artificially contaminating sterile wine with 4 different combination of mixed microorganisms, at the above established minimal-detectable 332 concentration each (10<sup>4</sup> CFU/mL) i.e. Mix A: S. cerevisiae, P. membranifaciens; Mix B: S. 333

334 *cerevisiae*, *L. brevis*, *P. membranifaciens*; Mix C: *S. cerevisiae*, *L. brevis*, *G. oxydans*, *C. stellata*; 335 Mix D: *S. cerevisiae*, *L. brevis*, *P. membranifaciens*, *P. anomala*, *G. oxydans*. The DNAs extracted 336 from each mixture were used as substrate for LATE-PCR reactions and the obtained amplicons 337 were used to separately hybridize the microarrays (Figure 5). The results obtained confirm that the 338 microarray allows in a specific manner the clear and specific detection of different spoilage 339 microorganisms directly from contaminated wines.

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### 342 **4. Discussion**

343 Commonly, microbial species present in wine are identified using conventional microbiological 344 approaches based on cultivation methods (Bester, Cameron, Toit, D, & Witthuhn, 2010). Unluckily, 345 cultivation is time-consuming and labour intensive (Fleet, 1993; Kopke et al., 2000) whereas 346 morphological and physiological tests are not always useful to identify and classify different 347 microorganisms (Hernán-Gómez, Espinosa, & Ubeda, 2000; Muyzer, 1999). Traditional culture 348 methods, based on biochemical and physiological characteristics, often lead to disappointing results 349 and misidentification (Van Der Vossen & Hofstra, 1996), whereas methods based on molecular 350 detection and identification are fast and reliable (Krisch et al., 2016). Many culture-independent 351 molecular methods allow analysis of total microbial DNA, isolated from mixed microbial 352 populations, in order to detect and identify single microbes in food ecosystems (Cocolin, 353 Alessandria, Dolci, Gorra, & Rantsiou, 2013; Ivey & Phister, 2011). Genetic fingerprinting of 354 complex microbial populations is, at present, used broadly to investigate the microbial ecology of 355 grape must fermentations (Nisiotou, Spiropoulos, & Nychas, 2007; Rantsiou et al., 2013; Urso et 356 al., 2008). Polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) assay 357 has been also employed, because of its capability to detect, identify individual species and produce 358 the overall profile of microbial populations (Cocolin et al., 2013). Although the above methods 359 demonstrated to be able in specifically detect several wine spoilage microbes, the availability of 360 quick and sensitive methods to simultaneously monitor the presence of both prokaryotic and 361 eukaryotic contaminant microorganisms is of crucial importance to reduce economical losses and to 362 ensure wine safety.

363 Even though the DNA microarray technology still detains for its application some cons, such as the 364 needing of extensive bioinformatic analysis, this methodology has several pros when compared to 365 other molecular approaches. DNA microarray is a molecular identification method by which DNA 366 probes, grouped and arrayed on a slide, allow simultaneous molecular identification and 367 characterization of many specific sequences in a single step (Southern, 2001). The detection system 368 of the signal provides that each DNA fragment in the sample specifically hybridize with the 369 oligonucleotide probes spotted on the slide in a known position. The power of this technology lies 370 mainly in the ability to analyze simultaneously a large number of DNA sequences in a single 371 sample and a high number of samples in a compact and relatively cheap device.

When analyzing food for microbial contamination, this approach provides the opportunity to obtain detailed information about the presence of contaminant species (Rasooly & Herold, 2008). Considering the high number of species of bacteria and ascomycetes that could potentially be responsible for wine alteration (Bartowsky, 2009; Krisch et al., 2016), a broad-spectrum detection system as microarray technology might be very useful.

377 The purposes of this research was to develop a method based on the application of bioinformatic, 378 biochemical and molecular protocol and to validate the use of a DNA microarray, produced during 379 this work, for the simultaneous detection and identification of spoilage yeast and bacteria after the 380 isolation of their DNAs directly from wine. Wine is a co-culture of many different microorganisms, 381 either prokaryotic and eukaryotic, for this reason we also checked whether the microarray could 382 identify multiple targets in a mixed sample. To achieve this goal, it was essential to develop a 383 protocol for the extraction of genomic DNA from mixtures of eukaryotes and prokaryotes from 384 wine. Total DNA isolated from complex food matrices contains large amounts of DNA from 385 different microbial groups (bacteria and yeasts) that have the potential to interfere with specific

386 amplification of particular DNA sequences (Chen, Wang, & Chen, 2008). The few protocols 387 available in literature are poorly applicable for the extraction of genomic DNA from wine due to the 388 presence of high concentrations of polyphenolic compounds, which severely interfere with the 389 subsequent enzymatic reactions of PCR gene amplification (García-Beneytez, Moreno-Arribas, 390 Borrego, Polo, & Ibáñez, 2002; Siret, Boursiquot, Merle, Cabanis, & This, 2000). For these reasons, 391 it was very important to optimize a protocol of genomic DNA extraction from wine with the aim of: 392 i) extracting in a single step genomic DNA from mixtures of eukaryotic and prokaryotic cells, ii) 393 achieving DNA yields sufficient to realize subsequent reactions of gene amplification, iii) obtaining 394 preparations of good quality genomic DNA.

Polymorphisms of sequences coding for ribosomal RNA (rDNA) were selected as barcode for the identification of bacterial species. In prokaryotes, the *locus* encoding rRNA contains the highly conserved three genes, 16S, 23S and 5S, separated by highly variable regions known as "internal transcribed spacers" or ITS (Ludwig & Schleifer, 1994).

399 The rDNA locus has been widely used for the identification of bacterial (Lebonah et al., 2014) and 400 fungal (Das & Deb, 2015) species because: i) its products are abundant (up 80% of total cellular 401 RNA), can be isolated and identified easily, ii) the rRNA genes sequences are highly conserved 402 facilitating amplification by PCR, iii) the presence of highly variable regions allows discrimination 403 of the different species (Olsen, Lane, Giovannoni, Pace, & Stahl, 1986); moreover the rDNA 404 sequences of many bacterial species are available in data banks. The spacer region 16S-23S of 405 rDNA has been widely used also for the identification of Bacillus anthracis (Nübel et al., 2004) and 406 Campylobacter (Keramas et al., 2003) by microarray. Yeasts characterization was achieved by 407 designing the oligonucleotide probes considering variations in the ITS region sequences according 408 to Leinberger and coworkers (2005).

In general, the DNA microarray designed in this study allows the identification of five species of lactic acid bacteria (belonging to the genera *Lactobacillus* and *Pediococcus*) and three species of acetic acid (belonging to genera *Acetobacter* and *Gluconobacter*) as well as nine species of yeasts, 412 all together representing the 'etiological cause' of major alterations in the wine industry (Comi, 413 2005). The data produced by this work have shown that: i) an efficient procedure to obtain good 414 quality DNA preparations, to be used as PCR-template form microbial mixture, was developed, ii) 415 the oligonucleotide probes, specific for each considered microorganism, recognize only their 416 specific target, with the exception of the L hilgardi oligo that had also a 100% match with L 417 buchneri and also with the wine-unrelated species L. parabuchneri, L. keferi and L. rapi; iii) the 418 microarray is able to detect the presence of yeasts, lactic and acetic acid bacteria at very low concentrations (10<sup>4</sup> CFU/mL). The probes produced are suitable to distinguish their own target 419 420 DNAs from other target DNAs present on the microarray (Liu, Mirzabekov, & Stahl, 2001, Liu et 421 al. 2001) giving signal of high intensity and absence of background noise. Our findings indicate that 422 the probes used are characterized by a discrimination capacity better than those previously reported 423 (Drobyshev et al., 1997; Yershov et al., 1996; Zheng, Alm, Stahl, & Raskin, 1996). However, to 424 discriminate two closely related species like L hilgardii and L. buchneri it will be important to test 425 additional probes that could target other regions of rDNA, such as that between 23S and 5S pre-426 rRNA. Other possible strategies to obtain increased specificity and sensitivity could consider the 427 use of PNA (peptide nucleic acids) as an alternative to DNA as probes (Weiler, Gausepohl, Hauser, 428 Jensen, & Hoheisel, 1997) or the preparation of longer probes (Relógio, Schwager, Richter, 429 Ansorge, & Valcárcel, 2002).

In conclusion, in this study for the first time the microarray methodology was applied for the simultaneous identification of different species of yeasts and bacteria directly from wine. The microarray developed is a novel tool, which not only allows the identification of the most representative species of the microbial community responsible for wine spoilage but also the investigation of population dynamics of indigenous wine yeast and bacteria populations. However, the number of possible secondary wine spoilage agents is higher than the microbial species considered in this investigation and it is likely to increase in the future, because of the identification of new spoilage microorganisms. Further studies will be required in order to expand progressively thespecific target range by adding other oligonucleotide probes specific for novel microbial species.

439

### 440 Acknowledgements

441 This research was partially supported by the Apulia Region in the framework of the Projects

- 442 NEWINE (Bando "Ricerca e sperimentazione in Agricoltura"; Project code PRS\_042), BIOTECA"
- 443 (Bando "Aiuti a Sostegno Cluster Tecnologici Regionali"; Project code QCBRAJ6) and IPROVISP
- 444 (Bando "Aiuti a Sostegno Cluster Tecnologici Regionali"; Project code VJBKVF4). Vittorio
- 445 Capozzi is supported by Fondo di Sviluppo e Coesione 2007-2013 APQ Ricerca Regione Puglia
- 446 "Programma regionale a sostegno della specializzazione intelligente e della sostenibilità sociale ed
- 447 ambientale FutureInResearch". The authors thank Mr. Giovanni Colella for the valuable technical
- 448 help and Prof. J. Smith for proofreading and valuable linguistic advice.
- 449

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# **Captions to figures**

Figure 1. Microarray analyses carried out using a specific target-DNA for each array. The
 oligonucleotide probes were deposited by a manual contact printing MicroCaster<sup>TM</sup> Arrayer
 (Whatman, Maidstone, UK). The schematic representation of the array used is reported.

Figure 2. Microarray analyses carried out using for each array a target-DNA specific to the
 organism designated at the indicated concentrations of DNA template. The oligonucleotide probes
 were deposited by a manual contact printing MicroCaster<sup>TM</sup> Arrayer (Whatman, Maidstone, UK).

Figure 3. Microarray analysis performed using for each array target-DNAs specific for different organisms in the following mixtures: (A) *S. cerevisiae*, *S. pombe*; (B) *S. cerevisiae*, *P. membranifaciens*, *L. brevis*; (C) *S. cerevisiae*, *C. stellata*, *L. brevis*, *G. oxydans*; (D) *S. cerevisiae*, *P. anomala*, *P. membranifaciens*, *L. brevis*, *G. oxydans*. The oligonucleotide probes were deposited by a manual contact printing MicroCaster<sup>TM</sup> Arrayer (Whatman, Maidstone, UK). The schematic representation of the array used is reported.

Figure 4. Microarray analyses performed using: (A) genomic DNA extracted from wine artificially
inoculated with a mixture of the following microorganisms: *A. aceti* LMG1261, *L. brevis*LMG11435, *S. cerevisiae* CBS1171; (B) not inoculated wine. The oligonucleotide probes were
deposited by robotic spotting SpotArray<sup>TM</sup>24 (Perkin Elmer, *Waltham, USA*). *The schematic representation of the array used is reported*.

Figure 5. Microarray analysis performed using genomic DNA extracted from wine artificially
inoculated with a mixture of the following microorganisms: (A) *S. cerevisiae, P. membranifaciens*;
(B) *S. cerevisiae, L. brevis, P. membranifaciens*; (C) *S. cerevisiae, L. brevis, G. oxydans, C. stellata*; (D), *S. cerevisiae, L. brevis, P. membranifaciens, P. anomala, G. oxydans*; (E) not
inoculated wine. The oligonucleotide probes were deposited by robotic spotting SpotArrayTM 24
(Perkin Elmer, Waltham, USA). The schematic representation of the array used is reported.

# **Table 1.** Microorganism strains utilized in this study.

Organism	Strain
YEASTS	
Saccharomyces cerevisiae	S288c
Zygosaccharomyces rouxii	CBS 732
Zygosaccharomyces bailii	GK02
Brettanomyces bruxellensis	CBS 72
Schizosaccharomyces pombe	972
Pichia membranifaciens	CBS 107
Pichia anomala	CBS 5759
Candida stellata	CBS 157
Hanseniaspora vineae	CBS 2171
LACTIC BACTERIA	
Lactobacillus plantarum	WCFS1
Lactobacillus brevis	ATCC 367
Lactobacillus hilgardii	ATCC 8290
Pediococcus damnosus	ATCC 29358
Pediococcus pentosaceus	SL4
ACETIC BACTERIA	
Gluconobacter oxydans	621H
Acetobacter aceti	DSM3508
Acetobacter pasteurianus	ATCC33445

YEASTS	
Saccharomyces cerevisiae	NC_001144.5, MF118616.1, MF118614.1, MF118613.1, MF118612.1, MF118611.1, MF118610.1, MF118609.1, MF118608.1, MF118606.1, F118605.1, MF118604.1, LC269189.1, KY693710.1, KY693708.1, KY315926.1, KY962551.1, KY962550.1, KY962549.1, KX434761.1, Y794751.1, LC215450.1, KY488348.1, CP011466.1, KY794729.1, X859535.1
Zygosaccharomyces rouxii	KY106065, KY106071.1, KY106069.1, KY106068.1, KY106066.1, KY106065.1, KY106064.1, KY106063.1, KY106062.1, KY106061.1, KX539236.1, KX539235.1, KX539234.1, KX539233.1, KJ507666.1, KM249341.1, LN849134.1
Zygosaccharomyces bailii	KJ433981.1, KY106027.1, KY106026.1, KY106023.1, KY106022.1, KY106020.1, KY076624.1, NR_138201.1, LN849135.1, KP241898.1, KP132936.1, JX458104.1, JX458102.1, JX458100.1
Brettanomyces bruxellensis	KY103308.1, KY103322.1, KY103321.1, KY103320.1, KY103319.1, KY103318.1, KY103316.1, KY103315.1, KY103313.1, KY103312.1, KY103311.1, KY103309.1, KY103307.1, KU729031.1
Schizosaccharomyces pombe	CU329672, KY105378.1, NR_121563.1, JQ726610.1, EU916982.1, AY251633.1, V01361.1, AB054041.1, Z19578.1
Pichia membranifaciens	KY104614.1, KY104651.1, KY104650.1, KY104628.1, KY104627.1, KY104625.1, KY104624.1, KY104622.1, KY104621.1, KY104620.1, KY104619.1, KY104618.1, KY104617.1, KY104616.1, KY104615.1, KY104613.1, KY104611.1, KY104610.1, KY104609.1, Y104608.1
Pichia anomala	KY105894.1, KY105890.1, KY105895.1, KY105895.1, KY105892.1, KY105890.1, KY105889.1, KY105888.1, KY105887.1, KY105886.1, KY105883.1, KY105882.1, KY105880.1, KY105877.1, KY105876.1, KY105875.1, KY105874.1, KY105873.1, KY105872.1, KY105871.1, KY105870.1, KY105867.1, KY105865.1
Candida stellata	KY102416.1, AY160766.1, AY188852.1
Hanseniaspora vineae	KY103580.1, KY693711.1, KY103584.1, KY103583.1, KY103582.1, KY103581.1, KY076611.1, NR_138203.1, KM384180.1, KM384177.1, KM384176.1, KM384175.1, FI231441.1, FI231440.1
LACTIC BACTERIA	
Lactobacillus plantarum	NC_004567, CP021501.1, CP017379.1, CP017374.1, CP017363.1, CP017354.1, CP018209.1, CP020816.1, CP020861.1, CP019348.1, CP019722.1, CP017406.1, CP018324.1, CP013149.1, CP017954.1, CP015308.1, CP013753.1, CP013749.1, CP016071.1, CP015857.1
Lactobacillus brevis	CP000416, CP005977.1, CP015398.1, AP012167.1, JN383920.1, JN368473.1, JN368472.1, JN368471.1, EF412991.1, EF412994.1, EF412993.1, EF412992.1, AY582720.1, AB102858.1, AY821851.1, AY839298.1, AF429617.1, AF429584.1, AF429583.1, AF429547., AF429542.1, AF405353.1, X74221.1
Lactobacillus hilgardii	NZ_GG670001.1, U161617.1, EF536365.1, EF536366.1, AJ616222.1, KU922755.1
Pediococcus damnosus	AF405365, AJ318414, CP012294.1, CP012288.1, CP012283.1, CP012275.1, CP012269.1, AF405385.1, AF405366.1, AF405376.1, AF405367.1
Pediococcus pentosaceus	NC_022780, CP015918.1, CP021474.1, CP006854.1, KC767943.1, JN696685.1, JN696705.1, CP000422.1
ACETIC BACTERIA	
Gluconobacter oxydans	CP000009, CP003926.1, CP004373.1, AB163823.1, AB163824.1, AB163830.1, AB163833.1, CP016328.1, AB163865.1, AB163861.1, AB163859.1, AB163841.1, AB163825.1
Acetobacter aceti	X74066, AB111902.1, AJ007831.1, AB161358.1, CP014692.1
Acetobacter pasteurianus	X71863, AJ007834, AJ007834.1, AB086017.1, AP014881.1, HF677570.1, AP011170.1, AP011163.1, AP011156.1, AP011149.1, AP011142.1, AP011135.1, AP011128.1, AP011121.1, AM049398.1

Table 2. Accession numbers of the sequences utilized to design primers and probes.OrganismSequence Acc. Nr.

681	
682	<b>Table 3.</b> Primer pairs for the specific amplification of the target
683	sequence of yeasts, lactic and acetic bacteria.
684	

Primer name	Primer sequence	Tm (°C)
YEASTS		
Liev_For	CAAGGTTTCCGTAGGTGAAC	58
Liev_Rev	CCAAGAGATCCRTTGYTGAA	58
LACTIC BACTERIA		
Latt_For	AACAAGGTAGCCGTAGGAGA	58
Latt_Rev	GTTAGTCCCGTCCTTCATCG	60
ACETIC BACTERIA		
Acet_For	TCGTAACAAGGTAGCCGTAG	58
Acet_Rev	CAAGCGTGTGCTCTAACCAA	60

Microorganism	Oligo sequence	Lenght	TM (°C)
VEASTS			
S. cerevisiae	ACTCTCCATCTCTTGTCTTCTTGCCCAG	28	70
Z. bailii	GAACACAACTACTCCAGACTCGTCAATC	28	68
Z. rouxii	CCCTCCAACACTTTGAGAGAACTCCGT	27	70
B. bruxellensis	TTATCCTTGCTTATCCACGTGTCTGCAC	28	68
S. pombe	TTCACAGAAAGGTAAATGGATAAGAGAAGAAA	32	66
P. membranifaciens	TGACGTGTGTATACTCCAGGTTTAGGTGTTT	31	70
P. anomala	TGTTTAGACCTTTGGGCAGTAAGCCAG	27	68
C. stellata	GACCGAAGTCTTGGCTGTTCACAGTGG	27	71
H. vineae	CGCGCAAACTACAGCCAATAGCAAGAAC	28	70
LACTIC BACTERIA			
L. plantarum	AACGGTAAATGCGATTAATGAGTTTAGCGATAA	33	68
L. brevis	TCAACAAGTATGTGTAGCCTCCGTATATTCCTT	33	70
L. hilgardii	GTTAACAAACTCAAAATAACGCGGTGTTCTCG	32	70
P. damnosus	CGACATATGTGTAGGTTTCCGTTTCTAAATATCC	34	70
P. pentosaceus	CCTACGGTAAAGTGATTAATTGAGTTTAGCG	31	68
ACETIC BACTERIA			
G. oxydans	AAATTATAGGAAGGGATATGTTGACGGCG	29	67
A. aceti	CAAACCCAGTCCAATCTGTGAGTTGAAA	28	67

AAACCCGACTGAATAACCTAGACAATACAT

**Table 4**. Oligo probes immobilized onto the epoxydated surface of the glass slide.

 A. pasteurianus



- 1 Gluconobacter oxydans
- 2 Pediococcus damnosus
- 3 Pichia membranifaciens
- 4 Acetobacter aceti
- 5 Pediococcus pentosaceus
- 6 Pichia anomala
- 7 Acetobacter pasteurianus
- 8 Zygosaccharomyces bailii
- 9 Candida stellata

- 10 Lactobacillus plantarum
- 11 Zygosaccharomyces rouxii
- 12 Hanseniaspora vineae
- 13 Lactobacillus brevis
- 14 Brettanomyces bruxellensis
- 15 Saccharomyces cerevisiae
- 16 Lactobacillus hilgardii
- 17 Schizosaccharomyces pombe

Figure 1



S. cerevisiae









G. oxydans









L. brevis

Figure 2



Figure 3



Figure 4

Figure 5 Click here to download high resolution image



1	Supplementary Materials
2	AN INNOVATIVE OLIGONUCLEOTIDE MICROARRAY TO DETECT SPOILAGE
3	MICROORGANISMS IN WINE
4	
5	Fabio Cimaglia <sup>1†</sup> , Mariana Tristezza <sup>2†</sup> , Antonietta Saccomanno <sup>3</sup> , Patrizia Rampino <sup>3*</sup> , Carla
6	Perrotta <sup>3</sup> , Vittorio Capozzi <sup>4</sup> , Giuseppe Spano <sup>4</sup> , Maurizio Chiesa <sup>1</sup> , Giovanni Mita <sup>2</sup> , Francesco
7	Grieco <sup>2*</sup>
8	
9	<sup>1</sup> Biotecgen, c/o Department of Biological and Environmental Sciences and Technologies,
10	University of Salento, 73100 Lecce, Italy.
11	<sup>2</sup> CNR – Institute of Sciences of Food Production (ISPA), via Prov. Lecce-Monteroni, 73100 Lecce,
12	Italy
13	<sup>3</sup> Department of Biological and Environmental Sciences and Technologies, University of Salento,
14	Lecce, Italy
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16	Italy
17	<sup>†</sup> Both authors contributed equally to this work
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25	patrizia.rampino@unisalento.it
26	

Table S1. Length of the ampliconsproduced after PCR assayrespectively using the Liev\_For/Liev\_Rev, Latt\_For/ Latt\_ Rev andAcet\_For/Acet\_Rev primer primes onyeasts, lactic and acetic bacteriagenomic DNA templates.

Microorganims	Lenght (bp)
YEASTS	
Saccharomyces cerevisiae	423
Zygosaccharomyces rouxii	287
Zygosaccharomyces bailii	426
Brettanomyces bruxellensis	154
Schizosaccharomyces pombe	483
Pichia membranifaciens	150
Pichia anomala	244
Candida stellata	187
Hanseniaspora vineae	350
LACTIC BACTERIA	
Lactobacillus plantarum	315
Lactobacillus brevis	326
Lactobacillus hilgardii	334
Pediococcus damnosus	342
Pediococcus pentosaceus	327
ACETIC BACTERIA	
Gluconobacter oxydans	212
Acetobacter aceti	280
Acetobacter pasteurianus	298



**Figura S1.** Electrophoretic profiles of amplification products of the chromosomal region corresponding to the gene cluster encoding the ribosomal RNA of bacteria (16S-ITS1) and yeasts (18S-5.8). The amplification was performed using the primers pairs *Acet\_For/Acet\_Rev* for acetic acid bacteria, *Latt\_For/Latt\_Rev* lactic acid bacteria and *Liev\_For/Liev\_Rev* for yeasts. Lane 1, *Gluconobacter oxydans*; lane 2, *Acetobacter pasteurianus*; lane 3, *A. aceti;* lane 4, *Lactobacillus plantarum*; lane 5, *L. hilgardii*; lane 6, *L. brevis*; lane 7, *Pediococus damnosus*; lane 8, *P. pentosaceus*; lane 9, *Brettanomyces bruxellensis*; lane 10, *Pichia membranifaciens*; lane 11, *Saccharomyces cerevisiae*; lane 12, *Zygosaccharomyces bailii*; lane 13, *Hanseniaspora vineae*; lane 14, *Pichia anomala*; lane 15, *Schizosaccharomyces pombe*; lane 16, *Z. rouxii*; lane 17, *Candida stellata;* lane M, DNA Ladder 100bp (Euroclone).

### 52 Figure S2.

Alignments

S. cerevisiae

### ACTCTCCATCTCTTGTCTTCTTGCCCAG

Download GenBank Graph				
	nics			
Saccharomyces cerevisiae str Sequence ID: KX891233.1 Length	rain LPBF3 small subun 1: 927 Number of Matches:	it ribosomal RNA 1	ene, partial sequence	
Range 1: 203 to 230 GenBank Grap	ohics	Ne	t Match Previous Match	
Score Expect 56.0 bits(28) 2e-05	Identities 28/28(100%)	Gaps 0/28(0%)	trand lus/Minus	
Ouery 1 ACTCTCCATCTCTTGTC	ITCTTGCCCAG 28			
Sbjct 230 ACTCTCCATCTCTTGTCT	TCTTGCCCAG 203			
Download GenBank Graph	nics			
Saccharomyces cerevisiae iso Sequence ID: MF118616.1 Length	blate Y27 internal transc n: 563 Number of Matches:	ribed spacer 1, pa 1	tial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spa	acer 2, partial sequence
Range 1: 18 to 45 GenBank Graphic	2	Ne	t Match Previous Match	
Score Expect 56.0 bits(28) 2e-05	Identities 28/28(100%)	Gaps 0/28(0%)	trand lus/Minus	
Query 1 ACTCTCCATCTCTTGTCTT Sbjct 45 ACTCTCCATCTCTTGTCTT	TCTTGCCCAG 28			
Sequence ID: MF118614.1 Length Range 1: 9 to 36 GenBank Graphics Score Expect	1: 640 Number of Matches:	1 Gaps	: Match Previous Match	and a partial bolic
56.0 bits(28) 2e-05 Query 1 ACTCTCCATCTTTGTCTT	28/28(100%) rcttgcccAg 28	0/28(0%)	/Minus	
56.0 bits(28)         2e-05           Query         1         ACTCTCATCTTGTCT           Sbjct         36         ACTCTCCATCTCTGTCT           Download         GenBank Graph           Saccharomyces cerevisiae isc         Sequence Ib: <u>MF1186131</u>	28/28(100%) rctracccaa 28 illiiiiiii rctracccaa 9 ilos vics vics vics vics vics vics vics vics vics vics vics vics victor vi	0/28(0%) scribed spacer 1, 1	us/Minus artial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed sp	pacer 2, partial sequenc
56.0 bits(28)         2e-05           Query 1         ACTICATIONS           Sbjct 36         ACTICATIONS           Download         GenBank Graph           Saccharomyces cerevisiae ics         Sequence ID: ME118613.1           Range 1: 10 to 37         Gmbank Graph	28/28(100%) rctTGCCCAG 28 incomparison incomparison incomparison rctTGCCCAG 9 incomparison in	0/28(0%) scribed spacer 1, 1	us/Minus artial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed sp	pacer 2, partial sequence
56.0 bits(28) 22-05 Query 1 ACTCCATCCATCTTOTT Sbjot 36 ACTCCATCTTOTT Download <u>GenBank Graph</u> Saccharomyces cerevisiae ist Sequence ID: <u>MF118613.1</u> Lengt Renge 11:10-b 37 <u>Gentlerk Graph</u>	28/28(100%) ITTGCCCAG 28 ITTTGCCCAG 9 NICS Value V25A internal trans is 680 Number of Matches: 28 28/28(100%)	0/28(0%) scribed spacer 1, 1 Keges 0/28(0%)	artial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed sp	pacer 2, partial sequenc
56.0 bits(28)         2e-05           Query 1         ACTCCCATCTTATCT           Sbjct 36         ACTCTCCATCTTATCT           Download         GenBank Graph           Saccharomyces cerevisiae is:         Sequence ID: MF18613.1 Length           Sequence ID: MF18613.1 Length         Second           Second         Expect           S6.0 bits(28)         2e-05           Sbic 1         ACTCTCCATCTTATCT           Sbjct 37         ACTCTCCATCTTATCT	28/28/28(100%) crtracccca 28 ilitititititi crtracccca 9 ilics is 80 Number of Matches: 3 Identities 28/28(100%) rctracccca 28 iritititititi rctracccca 19	0/28(0%) scribed spacer 1, 1 6aps 0/28(0%)	artial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed sp t Match Previous Match trand us/Minus	pacer 2, partial sequenc

Z. bailii

### GAACACAACTACTCCAGACTCGTCAATC

```
        Devices
        Contrast
        Contrast
```

Z. rouxii

### CCCTCCAACACTTTGAGAGAACTCCGT

Download Gen	Bank Graphics			
Saccharomyces so	genes for ITS1, 5.8S rRNA.	ITS2, 285 (RNA, 1	artial and complete sequen	e stran GY11349PS
Sequence ID: LC2729	09.1 Length: 696 Number of Mat	ches: 1		
Range 5: 39 10 65 Get	ntere doetros		And Party Prevents Party	
Score 54.0 bits(27)	Expect Identifies 6e-05 27/27(100%)	Geps 0/27(0%)	Strend Plus/Minus	
Durry 1 CERTICAN	CACTTYGAGAGAACTCCGT 27			
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Download Gen	Bank Graphica			
Zygosaccharomyce	es rouxii culture-collection CB	S:12631 small sub	unit ribosomal RNA gene, p	rtial sequence, internal transcribed spacer 1, 5.85 ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence
sequence to NU ISIN	ILL Langer / 20 Municle of Mar	Liter I		
Range 1: 62 to 68 Ger	Expect Identifies	Gaps	Strend	
\$4.0 bits(27)	6e-05 27/27(100%)	0/27(0%)	Diug/Minus	
Overy 1 CCCTCCAN	cactificataicacticut 27			
lbjci ## cccrccAd	ICACTITIGAGAGAACTCCGT 82			
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Sequence ID: KY1060	89.1 Length: 693 Number of Mat	shes: 1	ochuleu opaioler it, partier oed	zenze, si oo nuosunaa kinav gene anu aneman aanooneeu space 2, compete sequenze, ano aage suusala nuosunaa kinav gene, paruaa sequenze
Range 1: 36 to 53 G	dark Grafics		Not Not Name And	
Boore 54 0 hote(57)	Expect Identities	Gage 0/23/0843	Strand Ros Minut	
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SUSA 12 CONTERN	CACTTTGAGAGAACTCCOT 25			
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Zygosaccharomyce	es rouxil culture-collection CB	S:7804 internal tra	nscribed spacer 1, partial se	puence; 5.85 ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence
Sequence ID: KY1050	60.1 Length: 670 Number of Mat	utres: 1		
Range 1: 35 to 61 (at	Det Gastica	F 10.00	Same Parent Pressing Match	
54.0 bits(27)	64-05 27/27(100%)	0/27(0%)	Plus/Minus	
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SOUT AS CCCCCCA	CARTTEGAGAGAACTECOT 35			
Download Gen	Bank Scaphics			
Zygosaccharomyce Sequence ID: KY1060	es rouxil culture-collection CB 66.1 Langt: 650 Number of Mar	5.711 small subun stee: 1	t ribosomal RNA gene, parti	il sequence, internal transcribed spacer 1 and 5.85 ribosomal RNA gene, complete sequence, and internal transcribed spacer 2, partial sequence
Barren 1, 53 to 78 (m	desk Cardina		- International Address	
Score	Espect Identities	Gaps	Strand	
54.0 5t3(27)	64:05 27/27(100%)	0/27(0%)	Pius, Minus	
Query 1 CCCTCCAM	CACTTTGAGAGAGCTCCGT 27			
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57 58

### D. bruxellensis

### TTATCCTTGCTTATCCACGTGTCTGCAC

Alignments

Download GenBank Graphics			
Brettanomyces bruxellensis culture-collection CBS: Sequence ID: <u>KY103322.1</u> Length: 792 Number of Matche	2796 small sub	unit ribosomal RNA gene, p	partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;
Range 1: 74 to 101 GenBank Graphics		Next Match Previous Match	
Score         Expect         Identities           56.0 bits(28)         2e-05         28/28(100%)	Gaps 0/28(0%)	Strand Plus/Minus	
Query 1 TTATCCTTGCTTATCCACGTGTCTGGAC 28 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII			
Download GenBank Graphics			
Brettanomyces bruxellensis culture-collection CBS: Sequence ID: <u>KY103321.1</u> Length: 609 Number of Matche	74 small subun	it ribosomal RNA gene, par	tial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence;
Range 1: 212 to 239 GenBank Graphics		Next Match Previous Match	
Score         Expect         Identities           56.0 bits(28)         2e-05         28/28(100%)	Gaps 0/28(0%)	Strand Plus/Minus	
Query 1 TTATCCTTGCTTATCCACGTGTCTGCAC 28 Sbjct 239 TTATCCTTGCTTATCCACGTGTCTGCAC 212			
Download <u>GenBank</u> Graphics Brettanomyces bruxellensis culture-collection CBS:	98 small subun	it ribosomal RNA gene, par	tial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and
Sequence ID: <u>KY103320.1</u> Length: 469 Number of Matche	es: 1		
Range 1: 65 to 92 GenBank Graphics	6	Next Match Previous Match	
56.0 bits(28) 2e-05 28/28(100%)	0/28(0%)	Plus/Minus	
Query 1 TTATCCTTGCTTATCCACGTGTCTGCAC 28 Sbjct 92 TTATCCTTGCTTATCCACGTGTCTGCAC 65			
Download <u>GenBank Graphics</u> Brettanomyces bruxellensis culture-collection CBS:	75 small subun	it ribosomal RNA gene, par	tial sequence; internal transcribed spacer 1, 5 8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a
Sequence ID: <u>KY103319.1</u> Length: 477 Number of Matche	es: 1		
Range 1: 65 to 92 GenBank Graphics Score Expect Identities	Gans	Next Match Previous Match	
56.0 bits(28) 2e-05 28/28(100%)	0/28(0%)	Plus/Minus	
Query 1 TTATCCTTGCTATCCACGTGCTGCAC 28 Sbjct 92 TTATCCTTGCTATCCACGTGTCTGCAC 65			
Download <u>GenBank Graphics</u> Brettanomyces bruxellensis culture-collection CBS:	5206 internal tr	anscribed spacer 1. partial	sequence: 5.85 ribosomal RNA gene and internal transcribed spacer 2 complete sequence: and large subunit ribosomal RNA g
Sequence ID: KY103318.1 Length: 438 Number of Matche	es: 1		
Range 1: 28 to 55 GenBank Graphics Score Expect Identities	Gaps	Next Match Previous Match Strand	
56.0 bits(28) 2e-05 28/28(100%)	0/28(0%)	Plus/Minus	
Query 1 TTATCCTTGCTTATCCACGTGTCTGCAC 28 Sbjct 55 TTATCCTTGCTTATCCACGTGTCTGCAC 28			

Alignments

### C. pombe

### TTCACAGAAAGGTAAATGGATAAGAGAAGAAA

Download GenBank Graphics			
Schizosaccharomyces pombe culture-collection CB Sequence ID: KY105378.1 Length: 551 Number of Matche	S:1062 small st s: 1	ubunit ribosomal RNA ger	e, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence
Range 1: 206 to 237 GenBank Graphics		Next Match Previous Match	
Score Expect Identities 60.2 bits(32) 4e-07 32/32(100%)	Gaps 0/32(0%)	Strand Plus/Minus	
Query 1 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 32 Sbjct 237 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 206			
Download GenBank Graphics			
Schizosaccharomyces pombe ATCC 38366 ITS reg Sequence ID: <u>NR 121563.1</u> Length: 1077 Number of Mate	jion; from verifie	d material	
Range 1: 168 to 199 GenBank Graphics		Next Match Previous Match	
Score Expect Identities 60.2 bits(32) 4e-07 32/32(100%)	Gaps 0/32(0%)	Strand Plus/Minus	
Query 1 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 32 Sbjct 199 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 168			
Commode Centrating Visioning Schizosaccharomyces sp. UFLA CHYE5.39 185 rill Sequence ID: <u>JO728910.1</u> Length: 768 Number of Matche Range 1: 43 to 74 ( <u>centers</u> ) ( <u>inshize</u> ) <u>Score</u> Expect Identities <u>50.2</u> brs( <u>32</u> ) 44-07 32/32(100%) <u>Overv</u> 1 TYG/3/GAMOTABATGARAGAA3 32	Gaps 0/32(0%)	ne, partial sequence; inte Next Match Previous Match Strand Plus/Minus	rmal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA g
Sbjet 74 TTCACAGAAGGTAATGGATAAGAGAGAGA 43 Download <u>GenBank Graphics</u>	S ribonomal DN	A cape, partial equipped	internal transmitted encord 1, 5, 95 phonomal DNA page, and internal transmitted encord 2, complete excurption and 355 phonomal DI
Sequence ID: EU916982.1 Length: 1609 Number of Match	es: 1	A gene, partial sequence	internal nanschued spacer 1, 3.05 hudsonial KNA gene, and internal nanschued spacer 2, complete sequence, and 205 hudsonial KN
Range 1: 122 to 153 Genilank Graphics		Next Match Previous Match	
Score Expect Identities 60.2 bits(32) 4e-07 32/32(100%)	Gaps 0/32(0%)	Strand Plus/Minus	
Query 1 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 32 Sbjct 153 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 122			
Download <u>GenBank</u> <u>Graphics</u> Sort by: <u>E value</u>			
Schizosaccharomyces pombe chromosome III, com Sequence ID: <u>CU329672.1</u> Length: 2452883 Number of M	nplete sequence latches: 3		
Bange 1: 10320 to 10351 Genilank         Graphics           Score         Expect         Identities           60.2 bits(32)         4e-07         32/32(100%)	Gaps 0/32(0%)	Next Match Previous Match Strand Plus/Plus	
Query 1 TTCACAGAAAGGTAAATGGATAAGAGAAGAA 32 Sbjct 10320 TTCACAGAAAGGTAAATGGATAAGAGAAGAAA 103	51		
Ranne 2: 21190 in 21221 GeoBank Granhies	Next Match	Previous Match First Match	

### P. membranifacens

#### TGACGTGTGTATACTCCAGGTTTAGGTGTTT

GenBank Graphics Pichia membrasi Sustainati Pichia membrasinfaciens culture-collection CB5:636 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large s Sequence ID: <u>VY1048311</u> Length 701 Number of Matches: 1 
 Score
 Expect
 Identities

 60.0 bits(30)
 1e-06
 30/30(100%)
 Gaps Strand 0/30(0%) Plus/Minus Query 1 TGACGTGTGTATACTCCAGGTTTAGGTGTT 30 Sbjct 235 TGACGTGTGTATACTCCAGGTTTAGGTGTT 206 Download GenBank Graphics Pichia membranifaciens culture-collection CBS:1330 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large sequence ID: <a href="https://www.complete.com/www.complete.com/www.com/ww 
 Range 1: 220 to 249 [<u>Gentlenk</u> <u>Graphics</u>
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 Score
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 60.0 bits(30)
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 Query 1 TGACGTGTGTATACTCCAGGTTTAGGTGTT 30 Sbjct 249 TGACGTGTGTATACTCCAGGTTTAGGTGTT 220 Download <u>GenBank Graphics</u> Pichia membranifaciens culture-collection CBS:213 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5 & ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large s sequence (b: <u>Vide0821</u> Length: 488 Number of Matches: 1 <u>See 1 more title(s)</u> 
 Score
 Expect
 Identities

 60.0 bits(30)
 1e-06
 30/30(100%)
 Gaps Strand 0/30(0%) Plus/Minus Query 1 TGACGTGTGTATACTCCAGGTTTAGGTGTT 30 Sbjct 111 TGACGTGTGTATACTCCAGGTTTAGGTGTT 82 Download GenBank Graphics
Pichia membranifaciens culture-collection CBS:4707 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large
Sequence ID: KY104927.1
Length: 783 Number of Matches: 1 Range 1: 214 to 243 <u>Gentiank</u> <u>Graphics</u> Score Expect Identities 60.0 bits(30) 1e-06 30/30(100%) Gaps Strand 0/30(0%) Plus/Minus Query 1 TGACGTGTGTATACTCCAGGTTTAGGTGTT 30 Sbjct 243 TGACGTGTGTATACTCCAGGTTTAGGTGTT 214 Download GenBank Graphics Pichia membranificiens culture-collection CBS:184 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5 8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large s Sequence (b: <u>KY144263.1</u> Length 477 Number of Matches: 1 
 Big
 Range 1: 79 to 108 Gentlanic
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 Score
 Expect
 Identities
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 Strand

 60.0 bits(30)
 1e-06
 30/30(100%)
 0/30(0%)
 Plus/Minus
 Query 1 TGACGTGTGTATACTCCAGGTTTAGGTGTT 30 Sbjct 108 TGACGTGTGTATACTCCAGGTTTAGGTGTT 79

62 63

**Alignments** 

Alignments

### P. anomala

## TGTTTAGACCTTTGGGCAGTAAGCCAG

Download GenBank Graphics			
Wickerhamomyces anomalus isolate HN1 internal Sequence ID: <u>MF115993.1</u> Length: 615 Number of Match	transcribed spacer es: 1	1, partial sequence; 5.8S	ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence
Range 1: 111 to 137 GenBank Graphics		ext Match Previous Match	
Score Expect Identities 54.0 bits(27) 6e-05 27/27(100%)	Gaps 0/27(0%)	Strand Plus/Minus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 137 TGTTTAGACCTTTGGGCAGTAAGCCAG 111			
Download <u>GenBank</u> <u>Graphics</u> sort by: <u>E value</u> Wickerhamomyces anomalus strain CHY22 small s	▼ subunit ribosomal F	NA gene, partial sequenc	e; internal transcribed spacer 1 and 5 8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Sequence ID: KY626334.1 Length: 845 Number of Matche	es: 2		
Range 1: 441 to 467 GenBank Graphics Score Expect Identities	Gaps	Strand	
54.0 bits(27) 6e-05 27/27(100%)	0/27(0%)	Plus/Minus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 467 TGTTTAGACCTTTGGGCAGTAAGCCAG 441			
Range 2: 197 to 222 GenBank Graphics Score Expect Identities	Next Match	revious Match First Match	
38.2 bits(19) 3.4 26/2/(96%)	1/2/(3%)	Plus/Plus	
Sbjct 197 TGTTTAGACCTTT-GGCAGTAAGCCAG 222			
Download <u>GenBank Graphics</u> Sort by: E value	۲		
Wickerhamomyces anomalus strain STY20 small s	subunit ribosomal R	NA gene, partial sequenc	e; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Sequence ID: KY626333.1 Length: 919 Number of Match	es: 2		
Range 1: 461 to 487 GenBank Graphics Score Expect Identities	Gaps	Strand	
54.0 bits(27) 6e-05 27/27(100%)	0/27(0%)	Plus/Minus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 487 TGTTTAGACCTTTGGGCAGTAAGCCAG 461			
Range 2: 217 to 242 GenBank Graphics	Next Match	revious Match First Match	
Score Expect Identities 38.2 bits(19) 3.4 26/27(96%)	Gaps 1/27(3%)	Strand Plus/Plus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 217 TGTTTAGACCTTT-GGCAGTAAGCCAG 242	2, 27 (27.12)		
Download GenBank Graphics Sort by: Evalue	•		
Wickerhamomyces anomalus strain STY53 small s Sequence ID: KY626332.1 Length: 927 Number of Matchi	subunit ribosomal R es: 2	NA gene, partial sequenc	e; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Range 1: 466 to 492 GenBank Graphics	,	ext Match Previous Match	
Score Expect Identities 54.0 bits(27) 6e-05 27/27(100%)	Gaps 0/27(0%)	Strand Plus/Minus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 492 TGTTTAGACCTTTGGGCAGTAAGCCAG 466			
Range 2: 223 to 248 GenBank Graphics	Next Match	revious Match First Match	
score         Expect         Identities           38.2 bits(19)         3.4         26/27(96%)	Gaps 1/27(3%)	Strand Plus/Plus	
Query 1 TGTTTAGACCTTTGGGCAGTAAGCCAG 27 Sbjct 223 TGTTTAGACCTTT-GGCAGTAAGCCAG 248			

66 67

Alignments

C. stellata

### GACCGAAGTCTTGGCTGTTCACAGTGG

Download Ge	nBank Graph	ics			
Candida stellata Sequence ID: KY10	culture-collect 2416.1 Length:	tion CBS:157 small su : 468 Number of Matches	bunit ribosomal : 1	RNA gene, parti	tial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit
Range 1: 63 to 89	enBank Graphic	1		Next Match Previ	zvlous Match
Score 54.0 bits(27)	Expect 6e-05	Identities 27/27(100%)	Gaps 0/27(0%)	Strand Plus/Minus	
Query 1 GACCGA	AGTCTTGGCTGTT 	CACAGTGG 27          CACAGTGG 63			
Download Ge Candida stellata Sequence ID: AY160	nBank Graphi CBS 157 inter	ics rnal transcribed space : 432 Number of Matches	r 1, partial sequ	ience; 5.8S ribos	isomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Range 1: 51 to 77 (	enBank Granbin			Next Match Drevi	suface Matrix
Score 54.0 bits(27)	Expect 6e-05	Identities 27/27(100%)	Gaps 0/27(0%)	Strand Plus/Minus	
Query 1 GACCGA	AGTCTTGGCTGTT	CACAGTGG 27			
Download Ge	nBank Graphi	ics			
Candida stellata Sequence ID: AY18	strain CBS 15 3852.1 Length:	7 18S ribosomal RNA 365 Number of Matches	gene, partial s 1	equence; internal	al transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial st
Range 1: 1 to 24 Ge	nBank Graphics			Next Match Previ	zvlous Match
Score 48.1 bits(24)	Expect 0.003	Identities 24/24(100%)	Gaps 0/24(0%)	Strand Plus/Minus	
Query 1 GACCGA	AGTCTTGGCTGTT	CACAG 24       CACAG 1			

<u>Alignments</u>

H. vineae

56 0 hits(20) 20-05 20/00/100011	Gaps Stran	tch Previous Match d Minus	
36.0 Bits(28)         28-05         28/28(100%)           Query 1         CGCGCAAACTACAGCCAATAGCAAGAAC         28           Sbjct 105         CGCGCAAACTACAGCCAATAGCAAGAAC         78	0/28(0%) Plus/i	MINUS	
Download <u>GenBank Graphics</u> Hanseniaspora vineae culture-collection CBS:256	8 small subunit ribosomal	RNA gene, partial se	equence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, pa
Sequence ID: <u>KY103584.1</u> Length: 696 Number of Matcl Range 1: 132 to 159 <u>GenBank</u> <u>Graphics</u>	hes: 1 Next Mat	tch Previous Match	
Score         Expect         Identities           56.0 bits(28)         2e-05         28/28(100%)	Gaps Stran 0/28(0%) Plus/1	d Minus	
Query 1 COLOCAAACIALAGECTAAIAGEAAGAAC 28 Sbjet 159 CGCGCAAACTACAGGCAATAGCAAGAAC 132			
Download <u>GenBank Graphics</u> Hanseniaspora vineae culture-collection CBS:803 Sequence ID: KY(10358.1 Length: 812 Number of Matel	1 small subunit ribosomal	RNA gene, partial se	equence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, pa
Range 1: 239 to 266 GenBank Graphics Score Expect Identities	Next Mat Gaps Stran	tch Previous Match	
56.0 bits(28)         2e-05         28/28(100%)           Ouerv         1         CGCGCAAACTACAGCCAATAGCAAGAAC         28	0/28(0%) Plus/1	Minus	
Sbjct 266 CGCGCAAACTACAGCCAATAGCAAGAAC 239			
Download         GenBank         Graphics           Hanseniaspora vineae culture-collection CBS:277         Sequence ID: KY103582.1         Length: 819         Number of Materia	' small subunit ribosomal F hes: 1	RNA gene, partial seq	quence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, par
Range 1: 235 to 262 GenBank Graphics Score Expect Identities	Gaps Stran	tch Previous Match	
Db.U DITS(28)         Ze=05         28/28(100%)           Query 1         CGCGCAAACTACAGCCAATAGCAAGAAC         28	0/28(0%) Plus/I	MINUS	
Sbjet 262 CGCGCAAACTACAGCCAATAGCAAGAAC 235			
Download <u>GenBank Graphics</u> Hanseniaspora vineae culture-collection CBS:282 Sequence ID: KV103581.1 Jonathy 740. Number of March	7 small subunit ribosomal	RNA gene, partial se	equence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and la
Range 1: 135 to 162 GenBank Graphics	Next Mat	tch Previous Match	
Score         Expect         Identities           56.0 bits(28)         2e-05         28/28(100%)	Gaps Stran 0/28(0%) Plus/1	d Minus	
Query 1 CGCGCAAACTACAGCCAATAGCAAGAAC 28 Sbjct 162 CGCGCAAACTACAGCCAATAGCAAGAAC 135			
Developed Openhine			
actobacillus plantarum subsp. pla	ntarum isolate SF	RCM100434, c	complete genome
quence ID: CP021528 1 Length: 322			
equence ID: <u>CP021528.1</u> Length: 322	raphics		Next Match Previous Match
equence ID: <u>CP021528.1</u> Length: 322 inge 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect Ide</u> 2.1 bits(33) 2e-07 33	aphics entities /33(100%)	Gaps 0/33(0%)	Next Match Previous Match Strand Plus/Minus
Equence ID: <u>CP021528.1</u> Length: 322           inge 1: 323393 to 323425         GenBank         Gr           core         Expect         Ide           2.1 bits(33)         2e-07         33           ery 1         AACGGTAAATGGATAATG	raphics entities /33(100%) 5AGTTTAGCGATAA 33	Gaps 0/33(0%)	Next Match Previous Match Strand Plus/Minus
Equence ID: CP021528.1         Length: 322           ange 1: 323393 to 323425         GenBank         Gr           core         Expect         Idd           2.1 bits(33)         2e-07         33,           iery 1         AACGGTAAATGCGATTAATG         Idd           ijct 323425         AACGGTAAATGCGATT	aphics entities /33(100%) SAGTTTAGCGATAA 3: 	Gaps 0/33(0%) 3 23393	Next Match Previous Match Strand Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Ide 2.1 bits(33) 2e-07 33 lery 1 <u>AACGGTAAATGCGATTAATC</u> ijct 323425 <u>AACGGTAAATGCGATTAATC</u> lownload <u>GenBank Graphics</u> at he silve plantagements in SDC	aphics entities /33(100%) SAGTITAGCGATAA 3: AGTITAGCGATAA 3: Sort by: E value	Gaps 0/33(0%) 3 23393	Next Match Previous Match Strand Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect Ide</u> 2.1 bits(33) 2e-07 33, Iery 1 <u>ACCGTAAATGCGATTAATE</u> Ujct 323425 <u>AACGGTAAATGCGATTAATE</u> Download <u>GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325	aphics antities antities (33(100%) SAGTTTAGCGATAA 33 AGTTTAGCGATAA 33 Sort by: E value CM102022, comp (2258 Number of M	Gaps 0/33(0%) 3 23393 Dete genome latches: 5	Next Match Previous Match Strand Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Idi 2.1 bits(33) <u>2e-07</u> 33, IEry 1 <u>ACCGTAAATGCGATTAATG</u> if 232425 <u>AACGGTAAATGCGATTAATG</u> Jownload <u>GenBank Graphics</u> ctobacillus plantarum strain SRCC quence ID: <u>CP021501.1</u> Length: 325 nge 1: 499189 to 499221 <u>GenBank Grap</u>	aphics aphics entities (/33(100%)) SAGTTTAGCGATAA 32 SAGTTTAGCGATAA 32 Sort by: E value CM102022, comp i2258 Number of M raphics entities	Gaps 0/33(0%) 3 223393 lete genome latches: 5 Gaps	Next Match     Previous Match       Strand     Plus/Minus       Next Match     Previous Match       Strand     Strand
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> Gr core <u>Expect</u> Idd 2.1 bits(33) 2e-07 33, iery 1 <u>AACGGTAAATGCGATTAATGC UDVNIOAD GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 nge 1: 499189 to 499221 <u>GenBank</u> Gr ore <u>Expect</u> Idd .1 bits(33) 2e-07 33	aphics antities antit	Gaps 0/33(0%) 3 23393 23393 23393 23393 23393 20% 14tches: 5 Gaps 0/33(0%)	Next Match       Previous Match         Strand       Plus/Minus         Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> Gr core Expect Id 2.1 bits(33) 2e-07 33 iery 1 AACGGTAAATGCGATTAATG igt 323425 AACGGTAAATGCGATTAATG wwnload <u>GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 inge 1: 499189 to 499221 <u>GenBank</u> Gr ore Expect Id .1 bits(33) 2e-07 33 :ry 1 AACGGTAAATGCGATTAATG	aphics antities antities additional	Gaps 0/33(0%) 3 223993 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 23399 2339 2	Next Match       Previous Match         Strand       Plus/Minus         V       Next Match         Strand       Previous Match         Strand       Plus/Minus
Aquence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Id 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAATG</u> iery 1 <u>AACGGTAAATGCGATTAATG</u> Nownload <u>GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 nge 1: 499189 to 499221 <u>GenBank Graphics</u> ore <u>Expect</u> Id .:1 bits(33) <u>2e-07</u> <u>33</u> :ry 1 <u>AACGGTAAATGCGATTAATG</u> MACGGTAAATGCGATTAATG ict 499221 <u>AACGGTAAATGCGATTAATG</u> Nownload <u>GenBank Graphics</u> Sort	aphics antities antities antities addittraccGATAA 33 Sort by: E value CM102022, comp 2258 Number of M raphics entities y/33(100%) GAGTTTAGCGATAA 3 by: E value	Gaps 0/33(0%) 3 23393 0lete genome latches: 5 Gaps 0/33(0%) 33 199189	Next Match       Previous Match         Strand       Plus/Minus         Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Idd 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>ACC66TAAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGUS Cobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 ore <u>Expect</u> Idd .:1 bits(33) <u>2e-07</u> <u>33</u> iry 1 <u>AACCGGTAAATGCGATTAATGCGGTGAATGCGATTAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGATTAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGAATGCGGTGATGGGTGAATGCGGTGAATGCGGTGAATGCGGTGATGGGTGGG</u></u>	aphics antities	Gaps 0/33(0%) 3 22399 2239 223993 2239 22399 2239	Next Match       Previous Match         Strand       Plus/Minus         Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Idd 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAAT(</u> jct 323425 <u>AACGGTAAATGCGATTAAT(</u> )jct 323425 <u>AACGGTAAATGCGATTAAT(</u> )ownload <u>GenBank Graphics</u> ctobacillus plantarum strain SRCC quence ID: <u>CP021501.1</u> Length: 325 nge 1: 499189 to 499221 <u>GenBank Gr</u> ore <u>Expect</u> Idd :.1 bits(33) <u>2e-07</u> <u>33</u> :ry 1 <u>AACGGTAAATGCGATTAATG</u> jct 499221 <u>AACGGTAAATGCGATTAATG</u> it bits(33) <u>2e-07</u> <u>33</u> :ry 1 <u>AACGGTAAATGCGATTAATG</u> jct 499221 <u>AACGGTAAATGCGATTAATG</u> jct 499221 <u>AACGGTAAATGCGATTAATG</u> wunload <u>GenBank Graphics</u> sort :tobacillus plantarum strain TMW 1. uence ID: <u>CP017379.1</u> Length: 3141573 ge 1: 483650 to 483682 <u>GenBank Graphic</u>	aphics antities antit	Gaps 0/33(0%) 3 223993 22	Next Match       Previous Match         Strand       Plus/Minus         Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Idd 2.1 bits(33) 2e-07 33 IPY 1 AACGGTAAATGCGATTAATG if 232425 AACGGTAAATGCGATTAATG Download <u>GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 ore <u>Expect</u> Idd 1.1 bits(33) 2e-07 33 PY 1 <u>AACGGTAAATGCGATTAATG</u> if 499189 to 499221 <u>GenBank</u> <u>Gr</u> ore <u>Expect</u> Idd 1.1 bits(33) 2e-07 33 PY 1 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> Sort 1.1 bits(33) 2e-07 33 PY 1 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> Sort 1.1 bits(33) 2e-07 33/33 (ry 1 <u>AACGGTAAATGCGATTAATG</u> <b>GenBank Graphics</b> Sort 1.2 bits(33) 2e-07 33/33 (ry 1 <u>AACGGTAAATGCGATTAATG</u> <b>GenBank Graphics</b> Sort 1.2 bits(33) 2e-07 33/33 (ry 1 <u>AACGGTAAATGCGATTAATG</u>	aphics           aphics           aphics           aphics           aphics           aphics           aphics           aphics           aphics           staftTrACCGATAA           Sort by:           E value           CM102022, comp           c2258 Number of M           aphics           entities           aphics           daGTTTAGCGATAA           by:           E value           1623, complete ge           3 Number of Matchet           cs           ies         Ga           100%)         0/7           TAGCGATAA         33	Gaps         O/33(0%)         3           3         23393         23393           blete genome latches: 5         0         3           0/33(0%)         0/33(0%)         33           199189         •         •           enome s: 5         Nex         Nex           ps         S         S           33(0%)         P	Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322           ange 1: 323393 to 323425 <u>GenBank</u> <u>GenBank</u> <u>GenCanter</u> core         Expect         Idd         Idd <u>Ze-07</u> 33           iery 1         AACGGTAAATGCGATTAATG         AACGGTAAATGCGATTAATG <u>AacGGTAAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGTAATGCGATTA</u>	aphics antities	Gaps 0/33(0%) 3 223993 22	Next Match       Previous Match         Strand       Plus/Minus         Next Match       Previous Match         Strand       Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Idd 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAAT(</u> bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAAT(</u> bownload <u>GenBank Graphics</u> ctobacillus plantarum strain SRCC quence ID: <u>CP021501.1</u> Length: 325 inge 1: 499189 to 499221 <u>GenBank Graphics</u> iore <u>Expect</u> Idd 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAAT(</u> bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>AACGGTAAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGATTAATGCGATTAATGCGTTAATGCGTTAATGCGATTAATGCGATTAATGCGTTAATGCGATTAATGCGATTAATGCGTTAATGCGATTAATGCGTTAATGCGTTAATGCGATTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGATTAATGCGATTAATGCGTTAATGCGATTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGATTAATGCGATTAATGCGTAATGCGATTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGTTAATGCGATTAATGCGTTAA</u>	aphics aphics antities (33(100%) SAGTTTAGCGATAA 33 Sort by: E value CM102022, comp i2258 Number of M raphics entities (33(100%) GAGTTTAGCGATAA 33 Number of Matcher SS ies Ca (100%) 0/3 TTAGCGATAA 33 TTAGCGATAA 483650	Gaps 0/33(0%) 3 22399 223993 22399 223993 2239	Next Match       Previous Match         Strand       Plus/Minus
Aquence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Id 2.1 bits(33) <u>2e-07</u> <u>33</u> iery 1 <u>ACC6TAAATGCGATTAATC</u> by 1 <u>ACC6TAAATGCGATTAATC</u> by 1 <u>ACC6TAAATGCGATTAATC</u> by 1 <u>ACC6TAAATGCGATTAATC</u> by 1 <u>ACC6TAAATGCGATTAATC</u> is t 499189 to 499221 <u>GenBank</u> <u>Gr</u> ore <u>Expect</u> Id :1 bits(33) <u>2e-07</u> <u>33</u> if y 1 <u>ACC6TAAATGCGATTAATC</u> is t 499121 <u>AACGGTAAATGCGATTAATC</u> is t 499221 <u>AACGGTAAATGCGATTAATC</u> is t 493650 to 483682 <u>GenBank</u> <u>Graphic</u> is tobacillus plantarum strain TMW 1. <u>uence ID: CP017379.1</u> Length: <u>314157</u> ig 1: 483650 to 483682 <u>GenBank</u> <u>Graphic</u> is tobacillus plantarum strain TMW 1. <u>uence ID: CP017374.1</u> Length: 313208	aphics       aphics       antities       antities       y33(100%)       SAGTTTACCGATAA       SaGTTTACCGATAA       Sort by:       E value       CM102022, comp       2258       Number of M       raphics       entities       y/33(100%)       GAGTTTAGCGATAA       GAGTTTAGCGATAA       by:       E value       1623, complete ge       3 Number of Matches       Ga       TTAGCGATAA       TTAGCGATAA       Y:       E value       08, complete genoi       08, complete genoi	Gaps 0/33(0%) 3 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 223995 22395 22	Next Match       Previous Match         Plus/Minus
equence ID: <u>CP021528.1</u> Length: 322 ange 1: 323393 to 323425 <u>GenBank</u> <u>Gr</u> core <u>Expect</u> Id 2.1 bits(33) <u>2e-07</u> <u>33</u> Hery 1 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> ctobacillus plantarum strain SRC quence ID: <u>CP021501.1</u> Length: 325 nge 1: 499189 to 499221 <u>GenBank Graphics</u> ore <u>Expect</u> Id .:1 bits(33) <u>2e-07</u> <u>33</u> Hery 1 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> Sort it 499121 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> Sort it 499221 <u>AACGGTAAATGCGATTAATG</u> Download <u>GenBank Graphics</u> Sort it 483650 to 48362 <u>GenBank Graphics</u> ry 1 <u>AACGGTAAATGCGATTAATG</u> re <u>Expect</u> Identit <u>1 bits(33)</u> <u>2e-07</u> <u>33/33</u> (ry 1 <u>AACGGTAAATGCGATTAATGGATT</u> t 483650 to 48362 <u>GenBank Graphics</u> Sort <u>1 bits(33)</u> <u>2e-07</u> <u>33/33</u> (ry 1 <u>AACGGTAAATGCGATTAATGGATT</u> t 483682 <u>AACGGTAAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGGATT</u> t 483682 <u>AACGGTAAATGCGATTAATGGATT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGGAT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGAGT</u> t 483682 <u>AACGGTAAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTAATGCGATTATGAGT</u> t 483682 AACGGTAAATGCGATTAATGCGATTAATGCGATTAATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGATTATGCGAT	aphics antities (33(100%) SAGTTTAGCGATAA 33 Sort by: E value CM102022, complete geno (33(100%) GAGTTTAGCGATAA 4 (33(100%) GAGTTTAGCGATAA 4 by: E value 1623, complete geno 100%) 0/3 TTAGCGATAA 33 TTAGCGATAA 483659 (2) (2) (2) (2) (2) (2) (2) (2)	Gaps 0/33(0%) 3 223993 22	Next Match       Previous Match         Plus/Minus       Previous Match         Strand       Plus/Minus         wt Match       Previous Match         Strand       Plus/Minus
equence ID: CP021528.1       Length: 322         ange 1: 323393 to 323425       GenBank       Gr         core       Expect       Id         2.1 bits(33)       2e-07       33         uery 1       AACGGTAAATGCGATTAAT         optic 323425       AACGGTAAATGCGATTAAT         optic 10: CP021501.1       Length: 325         nge 1: 499189 to 499221       GenBank       Gr         ore       Expect       Id         ore       Expect       Id         ore       Expect       Id         ore       Expect       Identit         ict 499221       AACGGTAAATGCGATTAATGAAT       1.0         ownload       GenBank       Graphics       Sort         scalase2       AACGGTAAATGCGATTAATGAAT       1.0       1.0         iver       Expect       Identit       1.0       1.0         iver       Expect       Identit       1.0       1.0         iver       AACG	aphics aphics antities (33(100%) SAGTTTAGCGATAA 33 Sort by: E value CM102022, comp iz258 Number of M raphics entities (33(100%) GAGTTTAGCGATAA 4 CAGTTAGCGATAA 4 CAGTTAGCGATAA 33 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/33(0%) 3 23393 3 23393 3 23393 0/42 0/33(0%) 1 3 3 199189 ▼ 1 199189 ▼ 199189 ■ 199189 19918	Next Match       Previous Match         Plus/Minus       Previous Match         Strand       Plus/Minus         wt Match       Previous Match         Strand       Plus/Minus

<u>Alignments</u>

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# L. brevis

# TCAACAAGTATGTGTAGCCTCCGTATATTCCTT

Sequence ID: CP0	21674 1 Length	7440.370 Nilmone			
Range 1: 374866	to 374898 GanBan	k Granhics		Next Match	Previous Match
Score	Expect	Identities	Gaps	Strand	TIGHTER POLIT
62.1 bits(33)	2e-07	33/33(100%)	0/33(0%)	Plus/Minus	
Query 1 Sbjct 374898	TCAACAAGTATGTGTA 	GCCTCCGTATATTCCT	TT 33    TT 374866		
Download	GenBank Graph	CS Sort by: E V	alue	•	
Lactobacillus b Sequence ID: <u>CP(</u>	revis strain SR( )21479.1 Length	CM101174, com : 2411324 Numbe	plete genome er of Matches: 5		
Range 1: 94538 t	o 94570 <u>GenBank</u>	<u>Graphics</u>		Next Match	Previous Match
Score 62.1 bits(33)	Expect 2e-07	Identities 33/33(100%)	Gaps 0/33(0%)	Strand Plus/Min	us
Query 1 T Sbjct 94570 T	CAACAAGTATGTGTA 	GCCTCCGTATATTCCT 	TT 33     TT 94538		
Download (	GenBank Graphi	CS Sort by: E Va	alue	T	
Lactobacillus br	evis strain 100	08, complete ge 2351988 Numbe	enome er of Matches: 5		
Desired 101 01 0	- 27700 C - 2	Carabian		No. 4 March	Description Made
Score	Expect	Identities	Gaps	Next Match Strand	Previous Match
62.1 bits(33)	2e-07	33/33(100%)	0/33(0%)	Plus/Plu	JS
Query 1 Tr Sbjct 37758 Tr <i>L. hilga</i>	CAACAAGTATGTGTAC LIIIIIII CAACAAGTATGTGTAC urdii	CCTCCGTATATTCCT	33 37790 GTTAACAAA	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr <i>L. hilga</i> Download ~ <u>GenBy</u> Lactobacillus hilgar Sequence ID: <u>EU16161</u>	CAACAAGTATGTGTA( CAACAAGTATGTGTA( Urdii ank Graphics di strain ATCC 8290 7.1 Length: 834 Numbe	ICCTCCGTATATTCCT ILLILILICATATTCCT ICCTCCGTATATTCCT 16S-23S ribosomal R r of Matches: 1	GTTAACAAA	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr <i>L. hilga</i> BDownload ~ <u>GenBr</u> Lactobacillus hilgar Sequence ID: <u>EU16161</u> Range 1: 251 to 282 @ Score 60.2 bts(32)	CAACAAGTATGTGTA( CAACAAGTATGTGTA( Urdii ank Graphics dii strain ATCC 8290 7.1 Length: 834 Numbe Expect Identities 2e-12 32/32(100	In the second se	T 33 37790 GTTAACAAA RNA intergenic spacer and 2 V Next Match A Previous Match Strand Plus/Minus	CTCAAA	ATAACGCGGTGTTCTCC
Query 1 Sbjct 37758 T <i>L. hilga</i> Commond ~ General Lactobacillus hilgar Sequence ID: EUIGISI Range 1: 251 to 282 @ Score 60.2 bits(32) Query 1 Sbjet 282 GTTACAAA	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) CAACAAGTATGTGTA CAACAAGTATGTGTA CAACAAGTATGTGTGTCTCC	ICCTCCGTATATTCCT ICCTCCGTATATTCCT I6S-23S ribosomal R r of Matches: 1 Gaps 0/32(0%) 22 251	T 33 37790 GTTAACAAA NA intergenic spacer and 2 Next Match A Previous Match Strand Plus/Minus	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr L. hilga Bownload ~ GenBi Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 GTTACLAM (Download ~ GenBi Lactobacillus hilgar	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) di strain ATCC 8290 7,1 Length: 834 Numbe Dentary Greaplics Expect Jdentifie 2=12 32/32(100 CAAAATAACGCGTGTTCTCG CAAAAAGTATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT	IGCTCCGTATATTCCT	A 33 37790 GTTAACAAA RNA intergenic spacer and 2 RNA intergenic spacer	CTCAAA 23S ribosomal RN/	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr <i>L. hilggG</i> Bownload ~ GenEr Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 @ Score 60.2 bits(32) Query 1 CTTACCAAA @Download ~ GenEr Lactobacillus hilgar Sequence ID: EF53636	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) CAACATAACCCGFTCTCC CAACATAACCCGFTCTCCC CAAATAACCCGFTCTCCC CAAATAACCCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAAATAACCGFTCTCCC CAACTACGFTCTCCC CAAATAACCGFTCTCCC CAACTACGFTCTCCCCCCCCCCCCCCCCCCCCCCCCCCCC	INTERPOSE DESCRIPTION OF A CONTRACT OF A CON	T 33 T 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strand Plus/Minus	ACTCAAA 23S ribosomal RN/ ntergenic spacer, o	ATAACGCGGTGTTCTCC A gene, partial sequence
Query 1 Ti Sbjct 37758 Ti L. hilga Bownload ~ GenBi Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score Score Score Bojet 282 Otts(32) Query 1 TTAACAAA Bojet 282 OTTAACAAA Bojet 282 OTTAACAAA Score ID: EF556300 Score Score Sco	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( ank Graphics Case Construction CAACAAGTATGTGTA( CAACAAGTATGTATGTATGTA CAACAAGTATGTATGTATGTATGTA CAACAAGTATGTATGTATGTATGTATGTATGTATGTATGT	ISS-23S ribosomal R r of Matches: 1 0/32(0%) 22 281 20050mal RNA gene, p r of Matches: 1 Gaps posomal RNA gene, p	A 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strand Plus/Minus Dartial sequence; 16S-23S I Vext Match A Previous Match Strand	ACTCAAA 23S ribosomal RN/	ATAACGCGGTGTTCTCC
Query 1 T Sbjct 37758 T L. hilgGG Download ~ GenB: Lactobacillus hilgar Score 60.2 bits(32) Query 1 TTTACAMA Bjct 232 CTTACAMA Bjct 232 CTTACAMA Bownload ~ GenB: Lactobacillus hilgar Sequence ID: EFJSGA Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 CTTACAMA	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) dii strain ATCC 8290 7.1 Length: 834 Number Coast Caeptics Caacting Caeptics Caacting Caeptics CAACAAGTATGTCC CAACAAGCACGTGTTCCC CAACAAGCACGTGTTCCC CAACAAGCACGTGTTCCC CAACAAGCACGTGTTCCC CAACAAGCACGTGTTCCC	International RNA gene, p r of Matches: 1 Constant RNA gene, p	A 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strand Plus/Minus Next Match A Previous Match Strand Plus/Minus	ACTCAAA 23S ribosomal RN/	ATAACGCGGTGTTCTCC
Query 1 Sbjct 37758 L. hilgga Download ~ GenB: Lactobacillus hilgar Sequence ID: EUI631 Sbjct 251 to 282 G Score 60.2 bits(32) Query 1 CETSACAAA PDownload ~ GenB: Lactobacillus hilgar Sequence ID: EF58586 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 Sbjct 381 G Score 60.2 bits(32)	CAACAAGTATGTGTA( CAACAAGTATGTGTGTA( CAACAAGTATGTGTACGTGTGTCAGTAGTGTAGTGTAGT	CCTCCGTATATTCCT 111111111111111111111111111111111	A 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strand Plus/Minus Plus/Minus	CTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr L. hilga Bownload ~ GenBr Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 Gr Score 60.2 bits(32) Query 1 GTTACAAA Bownload ~ GenBr Lactobacillus hilgar Sequence ID: EF536367 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACAAA Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACAAA	CAACAAGTATGTGTA( CAACAAGTATGTGTACGTGTGTCCCCCCCCCCCCCCCCC	International RNA gene, p r of Matches: 1 Control RNA gene, p r of Matches: 1 Control RNA gene, p r of Matches: 1 Caps Postomal RNA gene, p r of Matches: 1 Caps Postomal RNA gene, p r of Matches: 1 Caps Postomal RNA gene, p Postomal RNA gene, p Post	A stand plus/Minus	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 L. hilga L. hilga Pownload ~ GenEs Lactobacillus hilgar Sequence ID: Ef13613 Gery 1 GTTAACAAA Download ~ GenEs Lactobacillus hilgar Secre 60.2 bits(32) Query 1 GTTAACAAA Construction Secre Secre Construction Secre Secre Construction Secre S	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) ank Graphics dii strain ATCC 8290 7.1 Length: 834 Numbe maak Graphics dii strain E112 168 ril 5.1 Length: 562 Numbe dii strain E91 168 rib 5.1 Length: 562 Numbe	16S-23S ribosomal R r of Matches: 1 2251 2551 2550 2551 2551 2551 2551 255	T 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match ▲ Previous Match Strand Plus/Minus Plus/Minus Artial sequence; 16S-23S in Plus/Minus	ACTCAAA 23S ribosomal RN/ ntergenic spacer, o	ATAACGCGGTGTTCTCC A gene, partial sequence
Query 1 T Sbjct 37758 T L. hilgGG Download ~ GenEy Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 TTTACLAA Comparison of the second Score 60.2 bits(32) Query 1 TTTACLAA Bojct 282 CTTACLAA Range 1: 356 to 381 G Score 60.2 bits(32) Query 1 TTTACLAA Sequence ID: EF536389 Range 1: 350 to 381 G Score	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA) di strain ATCC 8290 7.1 Length: 834 Numbe comment Graphics di strain E112 d68 ril 3.1 Length: 552 Numbe comment Graphics di strain E112 d68 ril 3.1 Length: 552 Numbe comment Graphics di strain E91 168 ril 5.1 Length: 562 Numbe comment Graphics di strain E91 168 ril 5.1 Length: 562 Numbe comment Graphics	ISC-TCCGTATATTCCT	T 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match ▲ Previous Match Strend Plus/Minus Plus/Minus Next Match ▲ Previous Match Strend Plus/Minus	ACTCAAA	ATAACGCGGTGTTCTCC A gene, partial sequence
Query 1 Tr Sbjct 37758 Tr L. hilga Bownload ~ GenB: Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 GTTACLAA Boyet 282 GTTACLAA Boyet 282 GTTACLAA Boyet 283 GTTACLAA Constraints of the state of the state Score 60.2 bits(32) Query 1 GTTACLAA Sequence ID: EF536369 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACLAA Bownload ~ GenB: Bownload ~ Gen	CAACAAGTATGTGTA( CAACAAGTATGTGTACAGTATGTGTACACGTGTGTGTACACGTGTGTGT	INTERPORT OF THE STREET	A 33 T 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strend Plus/Minus Plus/Minus A Previous Match Strend Plus/Minus A Previous Match Strend Plus/Minus	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr L. hilgGG Download ~ GenEr Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 TTTACLAM Bojct 282 GTTAACLAM Bojct 282 GTTAACLAM Bojct 282 GTTAACLAM Bojct 282 GTTAACLAM Bojct 281 GTTAACLAM Bojct 281 GTTAACLAM Bojct 281 GTTAACLAM Score 60.2 bits(32) Query 1 GTTAACLAM Bojct 381 GTTAACLAM Score 60.2 bits(32) Query 1 GTTAACLAM Score 60.2 bits(32) Query 1 GTTAACLAM	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC di strain ATCC 8290 7.1 Length: 834 Number CAAATAACCONFERENCE CAAATAACONFERENCE CAAATAACCONFERENCE CAAATA	Ites         Ites           Ites <td>A 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strend Plus/Minus A previous Match Strend Plus/Minus A previous Match Strend Plus/Minus</td> <td>ACTCAAA 23S ribosomal RN/ ntergenic spacer, c</td> <td>ATAACGCGGTGTTCTCC</td>	A 33 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match A Previous Match Strend Plus/Minus A previous Match Strend Plus/Minus A previous Match Strend Plus/Minus	ACTCAAA 23S ribosomal RN/ ntergenic spacer, c	ATAACGCGGTGTTCTCC
Query 1 L. hilgG Sbjct 37758 T L. hilgG Download ~ GenBi Lactobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 TTAACAAA Download ~ GenBi Sojet 381 GTTAACAAA Download ~ GenBi Socre 60.2 bits(32) Query 1 Sojet 381 GTTAACAAA Download ~ GenBi Socre 60.2 bits(32) Query 1 Sojet 381 GTTAACAAA Socre 60.2 bits(32) Query 1 Sojet 381 GTTAACAAA Socre 60.2 bits(32) Query 1 Sojet 381 GTTAACAAA Socre 60.2 bits(32) Query 1 Sojet 381 GTTAACAAA Socre 60.2 bits(32) Query 1 Socre 60.2 bits(32) Query 1 Socre Socre 60.2 bits(32) Query 1 Socre	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC CAACAAGTATGTGTAC distrain TATCC 8290 T_1 Length: 834 Number CAAAATAACCCOTTTCCC CAAAATAACCCOTTCTCC CAAAATAACCCOTTCTCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC CAAAATAACCCOTTCTCCC	International RNA gene, part of Matches: 1  Cost of Matches: 1  Co	A strand Plus/Minus Next Match A Previous Match Strand Plus/Minus Next Match A Previous Match Strand Plus/Minus Next Match A Previous Match Strand Plus/Minus Next Match A Previous Match Strand Plus/Minus	ACTCAAA 23S ribosomal RN/ ntergenic spacer, o	ATAACGCGGTGTTCTCC A gene, partial sequence
Query 1 L. hilgGC Download ~ GenBi Carbobacillus hilgar Sequence ID: EU16161 Range 1: 251 to 282 G Score 60.2 bits(32) Query 1 TTTACCMA Bojet 282 CTTAACCMA Comparison of the sequence ID: EF53638 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 CTTAACCMA Party 1 CTTAACCMA Bojet 282 CTTAACCMA Comparison of the sequence ID: EF53638 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 CTTAACCMA Range 1: 350 to 381 G Score Score 60.2 bits(32) Query 1 CTTAACCMA Range 1: 350 to 381 G Score	CAACAAGTATGTGTAK CAACAAGTATGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAG	ISC-TCCGTATATTCCT ISC-TCCGTATATTCCT ISC-TCCGTATATTCCT ISS-23S ribosomal R r of Matches: 1 ISS-23S ribosomal R ISS-23S ribosomal R	T 33 T 37790 GTTAACAAA RNA intergenic spacer and 2 Next Match ▲ Previous Match Strand Plus/Minus Plus/Minus artial sequence; 16S-23S in Plus/Minus Plus/Minus Plus/Minus Plus/Minus Strand Plus/Minus Strand Plus/Minus	ACTCAAA	ATAACGCGGTGTTCTCC
Query 1 Tr Sbjct 37758 Tr L. hilgG Bownload ~ GenBi Lactobacillus hilgar Sequence ID: EU16161 Bipt 222 GTTACAAA Gery 1 GTTACAAA Download ~ GenBi Lactobacillus hilgar Sequence ID: EF53636 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACAAA Download ~ GenBi Lactobacillus hilgar Sequence ID: EF53636 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACAAA Boownload ~ GenBi Lactobacillus hilgar Sequence ID: EF53636 Range 1: 350 to 381 G Score 60.2 bits(32) Query 1 GTTACAAA Download ~ GenBi Lactobacillus hilgar Sequence ID: EF53636 Range 1: 350 to 381 G Score 60.2 bits(32) Range 1: 350 to 381 G Score Boownload ~ GenBi Lactobacillus hilgar Sequence ID: AJ61627 Secore	CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTA( CAACAAGTATGTGTAC CAACAACAAGTATGTGTAC CAACAAGTATGTAC CAACAAGTATGTAC CAACAAGTATGTAC CAACAAGTATGTAC	Interference in the second sec	33     37790  GTTAACAAA  RNA intergenic spacer and 2  Next Match A Previous Match Strand Plus/Minus  artial sequence; 16S-23S in  Next Match A Previous Match Strand Plus/Minus  gene, strain DSM 20176 Next Match A Previous Match Strand	ACTCAAA 23S ribosomal RN/	ATAACGCGGTGTTCTCC A gene, partial sequence

	P. damnosus	CGACATATGTGTAGGTTTCCGTTTCTAAATATCC
	Pediococcus damnosus strain TMW 2.1536, complete Sequence ID: <u>CP012294.1</u> Length: 2125430 Number of Mate	e genome shes: 4
	Range 1: 189901 to 189934 GenBank Graphics	Next Match Previous Match
	Score         Expect         Identities           63.9 bits(34)         4e-08         34/34(100%)	Gaps Strand 0/34(0%) Plus/Plus
85	Query 1 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC 34 Sbjct 189901 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC 18	1 19934
	Download <u>GenBank Graphics</u> Sort by: <u>E value</u>	<u>■ ▼</u>
	Pediococcus damnosus strain TMW 2.1535, com Sequence ID: <u>CP012288.1</u> Length: 2247318 Number of	olete genome Matches: 4
	Range 1: 200860 to 200893 GenBank         Graphics           Score         Expect         Identities           63.9 bits(34)         4e-08         34/34(100%)	Gaps Strand 0/34(0%) Plus/Plus
86	Query 1 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC Sbjct 200860 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC	34 200893
	Download <u>GenBank Graphics</u> Sort by: <u>Evalue</u> Pediococcus damnosus strain TMW 2.1534, complet Sequence ID: <u>CP012283.1</u> Length: 2172287 Number of Ma	e genome tohes: 4
	Range 1: 140956 to 140989 GenBank Graphics	Next Match Previous Match
	Score         Expect         Identities           63.9 bits(34)         4e-08         34/34(100%)	Gaps         Strand           0/34(0%)         Plus/Plus
87	Query 1 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC 3 Sbjct 140956 CGACATATGTGTAGGTTTCCGTTTCTAAATATCC 1	4
	Download <u>GenBank Graphics</u> Sort by: <u>Evalue</u> Pediococcus damposus strain TMW 2 1533, comp	
	Sequence ID: <u>CP012275.1</u> Length: 2149374 Number of N	latches: 4
	Range 1: 44416 to 44449 GenBank Graphics	Next Match Previous Match
	Score Expect Identities 63.9 bits(34) 4e-08 34/34(100%)	Gaps Strand 0/34(0%) Plus/Minus
88	Query         1         CGACATATGTGTAGGTTTCCGTTTCTAAATATCC           Sbjct         44449         CGACATATGTGTAGGTTTCCGTTTCTAAATATCC	34 44416
89		
07		
	P. peniosaceus Download GenBank Graphics Sort by: E value	
	Pediococcus pentosaceus strain SRCM100892, cor Sequence ID: <u>CP021474.1</u> Length: 1785266 Number of Ma	nplete genome tiches: 5
	Range 1: 325609 to 325639 GenBank Graphics	Next Match Previous Match
	Score Expect Identities 58.4 bits(31) 1e-06 31/31(100%)	Gaps Strand 0/31(0%) Plus/Minus
90	Query 1 CCTACGGTAAAGTGATTAATTGAGTTTAGCG 31 Sbjct 325639 CCTACGGTAAAGTGATTAATTGAGTTTAGCG 325	589
	Download <u>GenBank</u> <u>Graphics</u> Sort by: <u>E value</u>	Ŧ
	Sequence ID: <u>CP015918.1</u> Length: 1739283 Number of Matches:	ne 5
	Score         Expect         Identities         Gat	Next Match Previous Match
	58.4 bits(31)         1e-06         31/31(100%)         0/           Output         1         CCTACCGTAAAGTGATTAATTGACTTAACTGACTG	31(0%) Plus/Plus
91	Sbjct 138082 CCTACGGTAAAGTGATTAATTGAGTTAGCG 130832	
	Pediococcus pentosaceus SL4, complete genome	¥
	Sequence ID: <u>CP006854.1</u> Length: 1789138 Number of Match	es: 5
	Score         Expect         Identities	Saps Strand
	_58.4 bits(31) 1e-06 31/31(100%) (	3/31(0%) Plus/Minus
	Sbjct 124954 CCTACGGTAAAGTGATTAATTGAGTTTAGCG 124924	
92	Download GenBank Graphics	
	Pediococcus pentosaceus strain CCUG32205 16S-2 Sequence ID: <u>KC767943.1</u> Length: 236 Number of Matches:	3S ribosomal RNA intergenic spacer and 23S ribosomal RNA gene, 1
	Range 1: 190 to 220 GenBank Graphics	Next Match Previous Match
	Store         Expect         Identities           58.4 bits(31)         1e-06         31/31(100%)	0/31(0%) Plus/Minus

Bownload - Ger	nBank Graphics	Sort by: E value	~			
Gluconobacter ox Sequence ID: LT900	xydans 621H iso 338.1 Length: 270	late WT-DSMZ ge 04625 Number of Ma	nome assembly, o tches: 235	chromosome: 1		
Range 1: 1264463 t	o 1264491 GenBa	nk Graphics	V Ne	xt Match 🛦 Previous Match		
Score 58.0 bits(29)	Expect 9e-10	Identities 29/29(100%)	Gaps 0/29(0%)	Strand Plus/Minus		
Query 1 Sbjct 1264491 Download ~ <u>Genf</u>	AAATTATAGGAAGG IIIIIIIIIIAAATTATAGGAAGG Bank <u>Graphics</u>	SATATGTTGACGGCG SATATGTTGACGGCG Sort by: E value	29 1264463 ~			
Gluconobacter oxy Sequence ID: <u>CP0043</u>	/dans DSM 3504 373.1 Length: 288	4, complete genon 2437 Number of Mar	ne tches: 265			
Range 1: 1381764 to	1381792 GenBan	<u>Graphics</u>	Vex Cases	t Match A Previous Match		
58.0 bits(29)	9e-10 2	9/29(100%)	0/29(0%)	Plus/Minus		
Sbjct 1381792 A	AATTATAGGAAGGG	ATATGTTGACGGCG 1	381764			-
Gluconobacter o Sequence ID: KF89	oxydans strain A 6258.1 Length: 6	uGo6 16S-23S rib 79 Number of Match	oosomal RNA inte nes: 1	rgenic spacer, partial s	equence	
Range 1: 105 to 13 Score	3 <u>GenBank</u> <u>Grap</u> Expect	hics Identities	Gaps	Next Match 🔺 Previous Ma Strand	tch	
58.0 bits(29) Query 1 AAA     Sbjct 133 AAA	9e-10 TTATAGGAAGGGAT IIIIIIIIIIIIIIIII TTATAGGAAGGGAT	29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIII ATGTTGACGGCG 105	0/29(0%)	Plus/Minus		
Download  Gluconobacter o Sequence ID: KE89	enBank <u>Graphics</u> oxydans strain A 6257 1 Length: 7	uGo11 16S-23S ri	ibosomal RNA inte	ergenic spacer, partial s	sequence	
Range 1: 113 to 14	1 GenBank Grap		103.1			
		hics	V 1	Next Match 🔺 Previous Ma	tch	
Score 58.0 bits(29) Query 1 AAA     Sbjct 141 AAA	Expect 9e-10 TTATAGGAAGGGAT 	hics Identities 29/29(100%) ATGTTGACGGCG 29             ATGTTGACGGCG 113	Gaps 0/29(0%)	Next Match 🔺 Previous Ma Strand Plus/Minus	tch	
Score 58.0 bits(29) Query 1 AAA III Sbjct 141 AAA A. d Download	Expect 9e-10 TTATAGGAAGGGAT IIIIIIIIIIIIIIIIIIIIIIII	hiss Identities 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIII ATGTTGACGGCG 113	Gaps 0/29(0%)	Next Match A Previous Ma	CCAATCTGT	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA Download ( Acetobacter act Sequence ID: AB1	Expect 9e-10 TTATAGGAAGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI GenBank Grap eti DNA, 16S-7 11902.1 Lengti	Lidentities 29/29(100%) ATGTTGACGGCG 29 11111111111 HTGTTGACGGCG 113 hics 23S rRNA ITS rg h: 900 Number of	Gaps 0/29(0%) B C. egion. strain:NB Matches: 1	Next Match A Previous Ma Strand Plus/Minus	CCAATCTGT	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA Download ( Acetobacter ace Sequence ID: AB1 Range 1: 192 to 2	Expect 9e-10 ITATAGGAAGGATI ITATAGGAAGGGATI ITATAGGAAGGGATI GenBank Grap eti DNA, 16S-1 11902.1 Lengti	hics 29/29(100%) ATGTTGACGGCG 29 111111111111 ATGTTGACGGCG 113 hics 23S rRNA ITS re h: 900 Number of phics	Gaps 0/29(0%) C. egion. strain:NB Matches: 1	Next Match A Previous Ma Strand Plus/Minus AAAACCCAGTC NRC 14818 Next Match	CCAATCTGT(	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA HII Sbjct 141 AAA A. ( Download ( Acetobacter act Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28)	Expect 9e-10 ITATAGGAAGGATI IIIIIIIIIIIIIIIIIIIIIIII	hics 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) C egion. strain:NB Matches: 1 Gaps 0/28(0	AAACCCAGTC	CCAATCTGT Previous Match	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA A. ( Download ( Acetobacter act Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28) Query 1 CAA, Sbjct 219 CAA	Expect 9e-10 TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT CONTRACTION TTATAGGAAGGGAT GenBank Grap eti DNA, 16S-7 11902.1 Lengtl 219 <u>GenBank Grap</u> Expect 2e-05 ACCCAGTCCAATCT	hics 29/29(100%) ATGTTGACGGCG 29 HILLIHIH HIGTTGACGGCG 113 hics 23S rRNA ITS re 23S rRNA ITS re 23S rRNA ITS re 24(28) 500 Number of phics Identities 28/28(100%) GTGAGTTGAAA 28 GTGAGTTGAAA 192	Gaps 0/29(0%) 3 egion. strain:NB Matches: 1 Gaps 0/28(0	Next Match A Previous Ma Strand Plus/Minus AAAACCCAGT( NRC 14818 Next Match Strand Plus/Minus	CCAATCTGT(	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA A. ( Download ( Acetobacter ace Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28) Query 1 CAA, Sbjct 219 CAA	Expect 9e-10 TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT GenBank Grap	hics Identities 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) 3 egion. strain:NB Matches: 1 Gaps 0/28(0	Next Match A Previous Ma Strand Plus/Minus AAAACCCAGTC NRC 14818 Next Match Strand 9%) Plus/Minus	CCAATCTGT(	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA Download ( Acetobacter ace Score 56.0 bits(28) Query 1 CAA Sbjct 219 CAA Download ( Acetobacter ace Score CAA Download ( Acetobacter ace Sequence ID: AJOI	Expect 9e-10 TTATAGGAAGGAT TTATAGGAAGGAT GenBank Grap eti DNA, 16S- 11902.1 Lengti 19 <u>GenBank Grap</u> Expect 2e-05 ACCCAGTCCAATCT ACCCAGTCCAATCT GenBank Grap eti internal trai 07831.1 Lengti	hics Identities 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) Gaps C. egion. strain:NB Matches: 1 Gaps 0/28(0 2 1 (ITS1), type s Matches: 1	AAACCCAGTC	CCAATCTGT(	GAGTTGAA
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Score 58.0 bits(29) Query 1 AAA Sbjot 141 AAA A. C Download S Acetobacter acc Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28) Query 1 CAA Sbjot 219 CAA Download S Acetobacter acc Sequence ID: AJ0 Range 1: 171 to 1 Score 56.0 bits(28) Query 1 CAA Sbjot 198 CAA	Expect 9e-10 TTATAGGAAGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI TTATAGGAAGGGATI GenBank Grap eti internal trai 07831.1 Lengti 98 GenBank Grap eti internal trai 07831.1 Lengti 98 GenBank Grap Expect 2e-05 ACCCAGTCCAATCT ACCCAGTCCAATCT ACCCAGTCCAATCT	hics 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) Gaps C. egion. strain:NB Matches: 1 Gaps 0/28(0 2 1 (ITS1), type s Matches: 1 Gaps 0/28(0	AAACCCAGTC Plus/Minus AAAACCCAGTC RC 14818 Next Match Strand Plus/Minus strain DSM 3508 Next Match Strand Plus/Minus	CCAATCTGTC	GAGTTGAA
Score 58.0 bits(29) Query 1 AAA Sbjct 141 AAA A. C Download G Acetobacter acc Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28) Query 1 CAA Sbjct 219 CAA Download G Acetobacter acc Sequence ID: AJ00 Range 1: 171 to 1 Score 56.0 bits(28) Query 1 CAA Sbjct 198 CAA	Expect 9e-10 TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT TTATAGGAAGGGAT COMPARIANCE	hics Identities 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) 3 egion. strain:NB Matches: 1 Gaps 0/28(0 2 1 (ITS1), type s Matches: 1 Gaps 0/28(0	AAACCCAGTC	CCAATCTGT(	GAGTTGAA
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Score 58.0 bits(29) Query 1 AAA Sbjot 141 AAA A. a Download ( Acetobacter acc Sequence ID: AB1 Range 1: 192 to 2 Score 56.0 bits(28) Query 1 CAA Download ( Acetobacter acc Sequence ID: AJ0 Range 1: 171 to 1 Score 56.0 bits(28) Query 1 CAA Download ( Acetobacter acc Sequence ID: AJ0 Range 1: 171 to 1 Score 56.0 bits(28) Query 1 CAA Sbjct 198 CAA Sbjct 198 CAA Download ( Acetobacter acc Sequence ID: AB1 Range 1: 260 to 2 Score Score	Expect 9e-10 TTATAGGAAGGAT TTATAGGAAGGAT TTATAGGAAGGAAGGAT TTATAGGAAGGAAGGAT TTATAGGAAGGAAGGAT TTATAGGAAGGAAGGAT COMPANY	Identities 29/29(100%) ATGTTGACGGCG 29 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Gaps 0/29(0%) Gaps 0/29(0%) C. egion. strain:NB Matches: 1 Gaps 0/28(0 2 1 (ITS1), type s Matches: 1 Gaps 0/28(0 1 1 23S rRNA ITS, a Matches: 1	AAACCCAGTO	CCAATCTGT(	GAGTTGAA

Acetobacter pasteul Sequence ID: <u>LN61314</u> See 1 more title(s) Range 1: 187 to 216 G Score 60.0 bits(30)	rianus geno 0.1 Length: enBank Graph	omic DNA containing 712 Number of Matche	g 16S-23S interg es: 1	jenic spacer reg	gion, isolate BJK_3B
Range 1: 187 to 216 G           Score           60.0 bits(30)           Ouery         1	enBank <u>Grap</u> i				
Score 60.0 bits(30)		nics		Next Match P	revious Match
Query 1 44400064	Expect 1e-06	Identities 30/30(100%)	Gaps 0/30(0%)	Strand Plus/Minus	
Sbjct 216 AAACCCGA		TAGACAATACAT 30             TAGACAATACAT 187			
Download GenB	ank <u>Graphi</u>	<u>CS</u>			
Acetobacter pasteur Sequence ID: <u>AB75459</u>	rianus DNA 1 <u>1.1</u> Length:	, 16S-23S ribosoma 784 Number of Match	I RNA intergeni es: 1	c spacer, partial	l sequence, strain: SL13E-2
See 1 more title(s)	Reality Correl			Nort Match	and the state
Score 60.0 bits(30)	Expect 1e-06	Identities	Gaps 0/30(0%)	Strand Dlus/Minus	revious Match
Sbjct 235 AAACCCCA Download <u>GenB</u> Acetobacter pasteu	ank <u>Graphi</u>	LAGACAATACAT 206 <u>cs</u> al 16S-23S internal	transcribed space	cer, ITS, strain I	FO 3283
Sequence ID: <u>AJ88887</u>	7.1 Length:	647 Number of Matche	es: 1	North Markether D	and the state
Score 60.0 bits(30)	Expect 1e-06	Identities 30/30(100%)	Gaps 0/30(0%)	Strand Plus/Minus	revious Match
Query 1 AAACCCGA          Sbjct 188 AAACCCGA	ACTGAATAACCT             ACTGAATAACCT	TAGACAATACAT 30                   TAGACAATACAT 159			
Download GenB	ank <u>Graphi</u>	<u>CS</u>			
Acetobacter pasteur Sequence ID: AJ00783	rianus inter 4.1 Length:	nal transcribed space 724 Number of Matche	er 1 (ITS1), type s: 1	e strain LMG 12	62
Range 1: 184 to 213 G	enBank Graph	nics		Next Match P	revious Match
Score 60.0 bits(30)	Expect 1e-06	Identities 30/30(100%)	Gaps 0/30(0%)	Strand Plus/Minus	
Query 1 AAACCCG/		TAGACAATACAT 30			

- 104 105 Figure S2. Evaluation of the specificity of the 17 species-specific oligoprobes by sequence alignment and
- similarity search carries out by BLAST. Each primer sequence and the source organism are indicated.



113 Figure S2. Panel A. Electrophoretic profiles of amplification 114 products of rDNA region 18S-5.8S from S. cerevisiae. The 115 amplification was performed using the pair of primers 116 *Liev\_For\_Cv5/Liev\_Rev* and different amounts of target-DNA: lane 117 118 1, 50 pg; lane 2, 10 pg; lane 3, 2 pg; lane 4, 0.4 pg; M, DNA Ladder 100 bp (Euroclone). Panel B. Electrophoretic profiles of 119 amplification products of rDNA region 16S-ITS1 from G. oxydans. 120 The amplification was performed using the pair of primers 121 Acet\_For\_Cy5/Acet\_Rev. and different amounts of target-DNA: 122 lane 1, 50 pg; lane 2, 10 pg; lane 3, 2 pg; lane 4, 0.4 pg; M, DNA 123 Ladder 100 bp (Euroclone). Panel C. Electrophoretic profiles of 124 amplification products of rDNA region 16S-ITS1 from L. brevis. 125 The amplification was performed using the pair of primers 126 Latt\_For\_Cy5/Latt\_Rev. and different amounts of target-DNA: 127 lane 1, 50 pg; lane 2, 10 pg; lane 3, 2 pg; lane 4, 0.4 pg; M, DNA 128 Ladder 100 bp (Euroclone). 129 130



Figure S3. Electrophoretic profiles of amplification products obtained by PCR multiplex of the chromosomal region corresponding to the gene cluster encoding the ribosomal RNA of bacteria (16S-ITS1) and yeasts (18S-5.8). The amplification was performed using the pairs of primers Acet\_For/Acet\_Rev for Latt\_For/Latt\_Rev lactic acid acetic acid bacteria, bacteria and Liev\_For/Liev\_Rev for yeasts. Lane 1, S. cerevisiae, S. pombe; lane 2, S. cerevisiae, P. membranifaciens, L. brevis; lane, S. cerevisiae, C. stellata, L. brevis, G. oxydans; lane 4, S. cerevisiae, P. anomala, P. membranifaciens, L. brevis, G. oxydans; lane M, DNA Ladder 100 bp (Euroclone).

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