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Detection of *Dekkera-Brettanomyces* Strains in Sherry by a Nested PCR Method

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***Brettanomyces* sp. and its ascosporogenous sexual state, *Dekkera* sp., have been well documented as spoilage microorganisms, usually associated with barrel-aged red wines. In this report, we describe the genetic characterization, on the basis of DNA content per cell, electrophoretic karyotyping, and mitochondrial DNA restriction patterns, of a *Dekkera* yeast strain isolated from sherries and of a number of other *Brettanomyces* and *Dekkera* strains. By using a genomic DNA fragment of the isolated *Dekkera* strain, we developed a two-step PCR method which directs the specific amplification of target DNA from this strain and from other *Brettanomyces-Dekkera* strains. The method efficiently amplified the target DNA from intact cells, obviating DNA isolation, and yielded a detection limit of fewer than 10 yeast cells in contaminated samples of sherry.**

Yeasts of the genera *Brettanomyces* and *Dekkera* (its sexual state) are some of the more troublesome yeasts that grow in fruit juice and wine. These microorganisms (and certain lactic acid bacteria) are responsible for the unpleasant odor and taste known as "mousiness" (7, 8) and cause economic losses that run into the hundreds of thousands of dollars (5). The classical habitat for both yeasts is barrel-aging red wine, but *Brettanomyces-Dekkera* yeasts have been isolated from many other fermented beverages, such as beer and cider (5, 6, 19). Therefore, efforts toward monitoring, isolation, and control of these species affect a large number of beverages.

Monitoring and control of these species is, however, difficult work, and part of the problem is the difficulty encountered in routine isolation and identification at the genus level. These microorganisms grow very slowly in current yeast media, and the rapid growth of other yeasts and bacteria makes the isolation step difficult (5, 14); in attempts to isolate minority populations of slowly growing *Brettanomyces-Dekkera* yeasts from mixed flora, cycloheximide is often included as a selective inhibitor to impede the growth of numerically superior microbes such as *Saccharomyces* sp. (cycloheximide sensitive). However, this property is also common to the genera *Hansenioaspora* and *Kloeckera*, for instance, which are also part of the normal flora of wine fermentation. *Brettanomyces-Dekkera* species are strongly acidogenic, producing large amounts of acetic acid from the oxidation of ethanol (5, 6). Thus, in addition to the cycloheximide-resistant character, production of acetic acid has also been used as a diagnostic test for the occurrence of *Brettanomyces* and *Dekkera* yeasts. The involvement of *Brettanomyces-Dekkera* species in wine spoilage has been reported in many different fermented beverages, but these microorganisms were not noted in sherries. These wines are made in Jerez, Spain, by a traditional procedure (3). The characteristic properties of sherries are due mainly to aging, which occurs in oak barrels under a film of *Saccharomyces* yeasts (known as "flor" yeasts) (11). This yeast film has two principal functions in the aging procedure; firstly, flor yeast metabolism greatly contributes to the particular characteristics of sherry, and secondly, the active consumption of oxygen and the isolation effect exerted by the yeast layer prevent wine oxidation. The reduced conditions (low oxygen concentration) produced by the flor yeast layer, together with the inhibitory concentration of ethanol (over 15%), confer, a priori, an inappropriate environment for the development of *Dekkera-Brettanomyces* strains in sherries. However, routine chemical analysis of sherries occasionally reveals the presence of abnormally high amounts of acetic acid in the absence of contaminating bacteria. We isolated yeast colonies from these acidified wines which, according to standard taxonomic criteria, belonged to the species *Dekkera bruxellensis* (1). In this report, we describe the genetic characterization of this *Dekkera* yeast and the development of a nested PCR method which successfully detects *Brettanomyces-Dekkera* strains.

MATERIALS AND METHODS

Strains and culture conditions. The strains used in this study and their origins are listed in Table 1. *Brettanomyces anomalus* (STCC1008, CBS77) and *B. clausenii* (STCC1011, CBS76) are synonyms for the *D. anomala* (STCC1008, CBS77) type strain; *B. bruxellensis* (STCC1009, CBS72) and *D. intermedia* (STCC1452, CBS4914) are synonyms for the *D. bruxellensis* (STCC1451, CBS74) type strain (2). *B. lambicus* (STCC1010, CBS75) is proposed in this study as a synonym for *D. bruxellensis. S. cheresiensis* (C7) and *S. montuliensis* (ET7) are synonyms for *Saccharomyces cerevisiae* (15). Strains C7 and ET7, as well as *Zygosaccharomyces rouxii* LA6 and *S. beticus* C5 and OSB138, are flor yeasts isolated from sherries.

Dekkera and *Brettanomyces* strains were grown on malt agar medium (2% malt extract, 2% glucose, 0.1% peptone, 2% agar) at 25°C. *Saccharomyces* and *Zygosaccharomyces* yeasts were grown on YPD medium (0.5% yeast extract, 1% peptone, 2% glucose, 2% agar) at 30°C.

Brettanomyces and *Dekkera* strains were obtained from the Spanish Type Culture Collection (STCC), Departamento de Microbiología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain. Flor yeasts were obtained from Osborne y Cia SA (OSB). Haploid strain *S. cerevisiae* MMY2 was kindly provided by R. Bailey and has been previously described (11).

Genetic characterization. Molecular karyotyping of the strains described in this work was performed with a contour-clamped homogeneous electric field system (Bio-Rad), essentially as described in reference 10. Mitochondrial DNA restriction analysis was carried out by following the procedure of reference 16, and flow cytometry analysis was carried out with a FACScan system (Becton-Dickinson) as described in reference 12.

Isolation of genomic DNA. A yeast cell pellet harvested by centrifugation of 100 ml of an overnight culture was suspended in 10 ml of CPES buffer (50 mM citrate-phosphate [pH 5.6], 40 mM EDTA [pH 8], 1.2 M sorbitol) with 10 mg of zymolyase 20 T and incubated at 37°C for 90 min. Treated cells were centrifuged for 7 min at 2,000 × *g*, suspended in 5.5 ml of TE (10 mM Tris HCl [pH 8], 1 mM EDTA [pH 8])–1% sodium dodecyl sulfate, and incubated at 65°C for 1 h. After incubation, 1.7 ml of 5 M potassium acetate was added and the mixture was incubated on ice for 5 min. This cell extract was centrifuged for 10 min at 5,000 × *g* and 4°C; 4 ml of supernatant was removed, mixed with an equal volume of isopropanol (ice chilled), and incubated for 20 min at −20°C. The mixture was

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TABLE 1. Strains used in this work

Species	Synonyms	Strain designation[a] used by:			Source
		OSB	STCC	CBS[b]	
Dekkera bruxellensis		OSB101			This study
Dekkera bruxellensis			STCC1451^T	CBS74^T	STCC
	Brettanomyces bruxellensis		STCC1009	CBS72	STCC
	Dekkera intermedia		STCC1452	CBS4914	STCC
	Brettanomyces lambicus		STCC1010	CBS75	STCC
Dekkera anomala	*Brettanomyces anomalus*		STCC1008^T	CBS77^T	STCC
	Brettanomyces claussenii		STCC1011	CBS76	STCC
Saccharomyces beticus		C5^F			OSB
Saccharomyces beticus		OSB138^F			OSB
Saccharomyces cerevisiae		MMY2			R. Bailey
	Saccharomyces cheresiensis	C7^F			OSB
	Saccharomyces montuliensis	ET7^F			OSB
Zygosaccharomyces rouxii		LA6^F			OSB

[a] T, type strain; F, flor yeast.
[b] Centraalbureau voor Schimmelcultures Yeast Division.

centrifuged, and the pellet was washed with 70% ethanol and resuspended in 0.25 ml of TE. This solution was phenol-chloroform extracted, and RNA was removed by RNase A treatment. One-tenth of a milliliter of sodium acetate (final concentration, 0.3 M) and 1 ml of ethanol were added to precipitate the DNA. The precipitated DNA was washed with 70% ethanol and finally resuspended in 50 μl of TE.

Library construction. DNA from *D. bruxellensis* OSB101 was digested with *Hin*dIII, the digested DNA was ligated to the pBluescriptSK cloning vector (Stratagene), and chimeric plasmids containing a DNA fragment from strain OSB101 were selected (17). The DNA sequence of both strands of one of these fragments, chosen to design appropriate PCR primers, was determined by using an ALF sequencer (Pharmacia) and the sequencing kit provided by Pharmacia.

Dot and slot blot hybridization. Genomic DNA samples (200 ng) from yeast strains and salmon sperm were transferred to a hybridization membrane by dot blotting or slot blotting in a Hoefer slot blot system. Subsequent hybridization was performed as previously described (4). A random primer method was used for radioactive labelling of the DNA proof (17).

PCR amplifications. Primers were purified by fast protein liquid chromatography (Pharmacia). The sequences of the primers used were as follows: DB1, AGAAGTTGAACGGCCGCATTTGCAC (sense); DB2, AGGATTGTTGAC ACTCTCGCGGAGG (antisense); DB3, CGGCATATCGAAGACAG (sense); DB4, CATCCTCGCCATACAAC (antisense). In the two-step PCR method designed in this work, primers BD1 and BD2 were used in the first reaction. The reaction mixture was preheated for 5 min at 95°C, and then 30 cycles were carried out, each of which consisted of a denaturing step (30 s, 95°C), an annealing step (30 s, 55°C), and an extension step (1 min, 72°C). After the last cycle, extension was continued for a further 10 min to allow completion of the reaction. The PCR mixture contained either intact cells, plasmid DNA, or 20 ng of genomic DNA (as indicated in Results and Discussion); 25 pmol of each oligonucleotide primer; each deoxynucleoside triphosphate at 0.2 mM; 10 μl of 10× *Taq* DNA polymerase buffer; and 2.5 U of *Taq* DNA polymerase, was made up to a total volume of 100 μl with sterile distilled water, and was overlaid with 100 μl of mineral oil. A 1-μl sample of this PCR-amplified DNA was used to initiate the second reaction (BD3 and BD4 were used as nested primers), which was achieved under conditions identical to those used for the first PCR, except that 65°C was used as the annealing temperature.

RESULTS AND DISCUSSION

Genetic characterization of a *Dekkera* yeast strain isolated from sherry. Sherries have a high content of ethanol (more than 15%) and a low-oxygen environment. These are adverse conditions for the development of many spoilage microorganisms that may proliferate in other types of wine (11). Our routine chemical analysis occasionally led to the discovery of barrels containing up to 1 mg of acetic acid per ml (five times more than usual). In many cases, no bacterial colonies were isolated; instead, slowly growing yeast colonies were recovered when a sample of the spoiled wine was plated. Cycloheximide resistance, production of acetic acid, and other properties described in taxonomic guides (1) suggested that the isolated yeast was *D. bruxellensis*. This yeast was able to produce acetic acid in malt extract medium and also acidified sterile sherry (data not shown), indicating that the yeast was responsible for the high acetic acid content (and probably other disagreeable characteristics) perceived in the acidified wine. *Brettanomyces* or *Dekkera* strains in sherry had not been previously described; for this reason, we first performed a comparative study of the electrophoretic karyotypes, the mitochondrial DNA restriction patterns, and the DNA contents per cell of the isolated yeast strain and a number of *Brettanomyces* and *Dekkera* strains.

As shown in Fig. 1a, the molecular karyotype of the isolated yeast was very similar to that of *Dekkera-Brettanomyces* type strains, all of which show four large chromosomes. In all cases, the relative amount of DNA was higher in some bands than in others, suggesting that different chromosomes are similar in size or that certain chromosomes are present in higher numbers than others, reflecting an aneuploid condition. The amounts of DNA per cell obtained in all of the *Brettanomyces* and *Dekkera* strains used varied from 0.9 to 1.8 times that found in haploid *S. cerevisiae* MMY2 cells (Fig. 1b). If haploid genomes of *Brettanomyces-Dekkera* and *Saccharomyces* yeasts are similar in size (similar DNA contents), the amount of DNA found in *Brettanomyces* and *Dekkera* yeasts would also suggest different degrees of aneuploidy in these natural yeasts. The *Dekkera* sp. strain OSB101 isolated showed the larger amount of DNA per cell (1.8-fold), a value more similar to that found in *B. bruxellensis* (1.5-fold) than to that of any other *Dekkera* strain. The smaller amount corresponded to *B. anomalus* and *B. claussenii* (0.9-fold), both of which have DNA content similar to that of haploid *Saccharomyces* yeasts.

A rapid and simple method of *Saccharomyces* yeast characterization, based on mitochondrial DNA restriction analysis, has been described for monitoring of wine fermentation (16). We applied this method to *Dekkera* and *Brettanomyces* yeasts, and interestingly, the procedure yielded characteristic mitochondrial DNA patterns in these yeasts as well (Fig. 1c). Mitochondrial DNA restriction analysis by this method indicated that the pattern observed in *Dekkera* sp. strain OSB101 was very similar to that of *D. bruxellensis* (Fig. 1c). *B. bruxellensis* and *B. lambicus* shared some bands with *Dekkera* strains (in particular, with OSB101), which could be indicative of a close phylogenetic relationship between these strains and *Dekkera* sp. strain OSB101 too. In general terms, the mitochondrial DNA restriction analysis patterns of different *Dekkera-Brettanomyces* strains which are closely related varied (Fig. 1c), indicating that the mitochondrial DNA is polymorphic in these

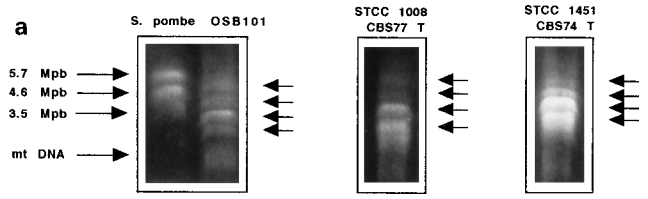


FIG. 1. Genetic characterization of *Dekkera* strain OSB101, isolated from sherry, in comparison with other *Dekkera* and *Brettanomyces* strains. (a) Electrophoretic karyotype of the *Brettanomyces* (STCC1008, CBS77) and *Dekkera* (STCC1451, CBS74) type strains, compared with that of *Dekkera* strain OSB101, isolated from sherry. *Schizosaccharomyces pombe* was used as the standard for chromosome size. (b) DNA contents per cell obtained by cytometric analysis of the strains indicated. Each arrow indicates the value (arbitrary units) obtained for nonbudded cells of each strain. (c) Restriction analysis of the mitochondrial DNA of the strains indicated digested with *Alu*I and *Hin*fI (*Hin*dIII-digested λ DNA was used as the standard). Mpb, megabase pairs. mt, mitochondrial.

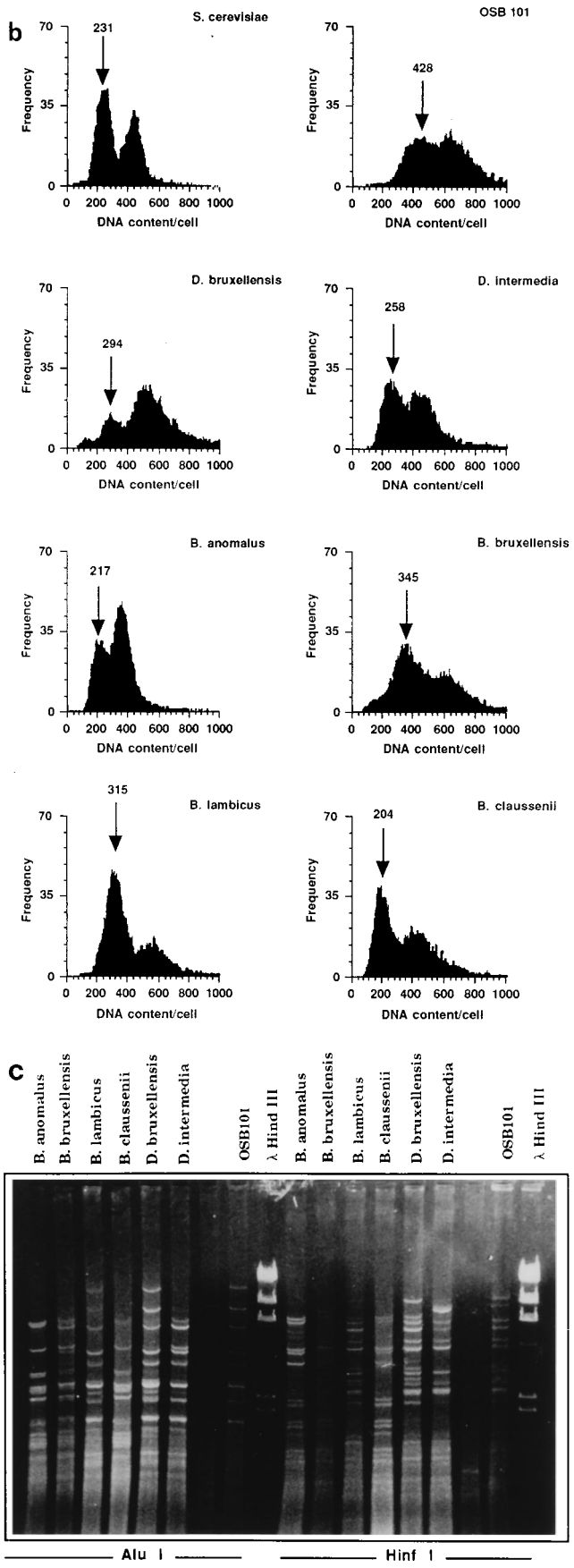
strains, as happens in *Saccharomyces* strains; however, the degree of polymorphism is probably lower, since two different strains isolated from completely different sources (*B. anomalus* and *B. claussenii*) had identical patterns.

Thus, on the basis of classical taxonomic guides and the above-described genetic characterization, we conclude that the acidifying yeast isolated in sherry actually belongs to the genus *Dekkera*. The similarity of *B. lambicus* and *D. bruxellensis* found in our study (for example, they have identical mitochondrial DNA patterns) also suggests that *D. bruxellensis* is the type strain of *B. lambicus*.

Detection of intact *Dekkera-Brettanomyces* yeast cells by nested PCR. The time frame for maximal cell number development and subsequent decline of *Brettanomyces-Dekkera* strains probably depends on wine chemistry and other environmental factors. In barrel-aging sherry, the high alcohol content and the anaerobic conditions conferred by the flor yeasts should prevent proliferation of these yeasts. In fact, occurrence of *Dekkera* species in sherry was occasional; however, in certain cases these yeasts became established and were capable of attaining substantial population densities. Therefore, programs monitoring the presence of *Dekkera* yeasts in sherry are also essential. The monitoring method of choice generally involves collection of microbes by membrane filtration and subsequent culture by using media designed to identify acetic acid-producing, cycloheximide-resistant yeasts. This method takes too long for identification, and in fact, alternatives such as enzyme-linked immunosorbent assays have been proposed (14).

The PCR is another reliable, sensitive, and rapid method capable of widespread application in processes such as wine production. The PCR procedure allows specific detection of DNA sequences, and thus it is ideally suited to the detection of contaminating microorganisms (9, 13, 18). To design a PCR protocol for specific detection of *Dekkera* species in wine, we initiated the search for a suitable DNA sequence in current databases, but the selection of such a sequence was restricted by the limited available gene data about these species. For this reason, we isolated and sequenced a DNA fragment from this yeast that is suitable for specific PCR amplification. To this end, we prepared a genomic library of the *Dekkera* sp. strain OSB101 yeast isolated from sherry (see Materials and Methods). Twenty clones of this library were randomly chosen, and three of them were further characterized as follows.

Saccharomyces species are the most abundant yeasts present in sherry. To prevent nonspecific amplification from these numerically superior strains, each *Dekkera* DNA fragment was



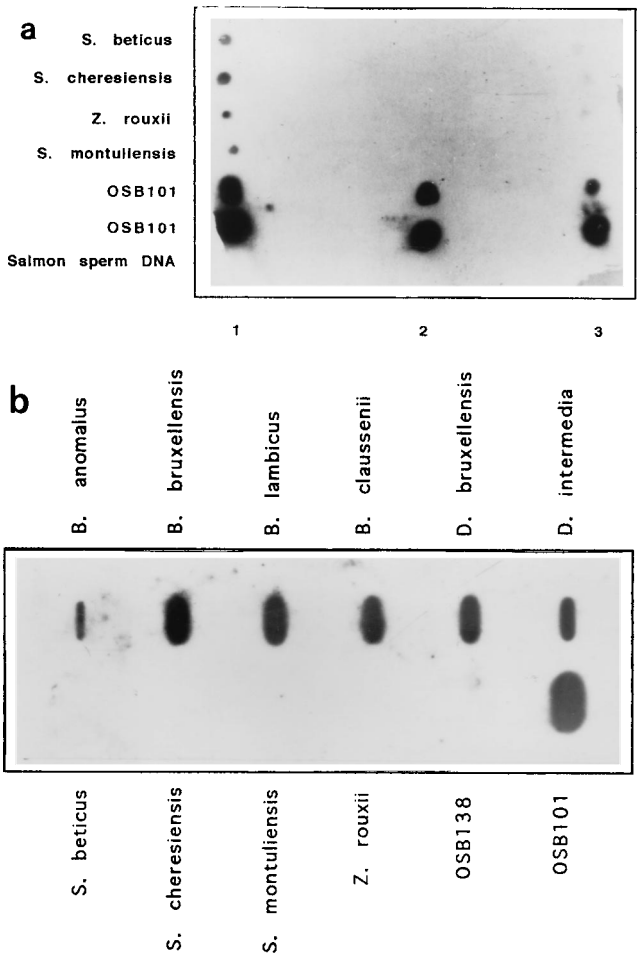


FIG. 2. (a) Dot blot analysis of genomic DNA isolated from the strains indicated, and probed with three radiolabelled DNA fragments from *D. bruxellensis* OSB101. (b) Slot blot analysis of DNAs from a variety of *Dekkera* and *Brettanomyces* strains and flor yeast strains (see Table 1) as a control, probed with the *D. bruxellensis* OSB101-specific hybridizing DNA fragment.

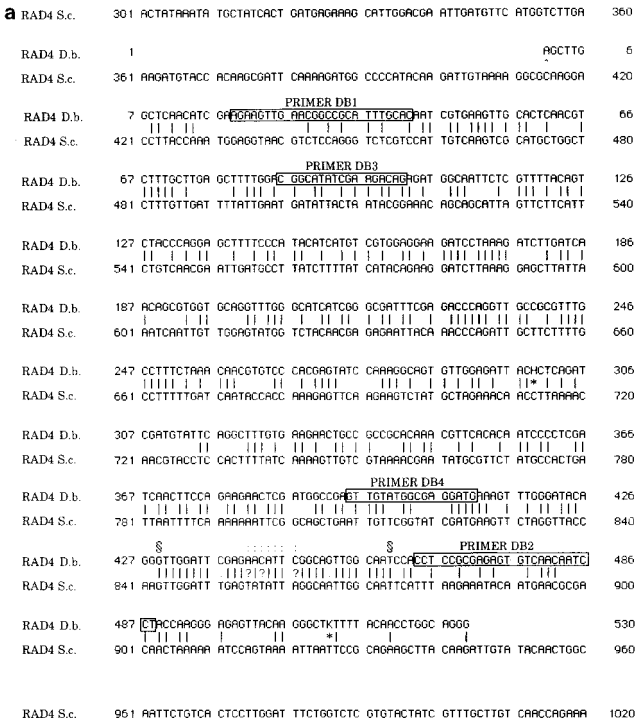


FIG. 3. (a) DNA sequence of the *Dekkera-Brettanomyces* (D.b.)-specific fragment and its homology to the *RAD4* gene of *S. cerevisiae* (S.c.). Sequences chosen for PCRs are in boxes. (b) Schematic representation of the nested PCR employed and DNA fragments produced by the first and second PCRs (molecular weights indicated are based on an *Hae*III-digested ϕ DNA standard). pb, base pairs.

radiolabelled and used to probe DNAs from *Dekkera* strain OSB101 and different flor yeast strains by dot blot analysis. Two of the radiolabelled probes cross-hybridized to the flor yeast DNA used, but one was specific for the DNA isolated from the *Dekkera* strain (Fig. 2a). This *Dekkera*-specific DNA fragment was also used to probe DNAs from different *Dekkera* and *Brettanomyces* strains, and the levels of cross-hybridization found were similar in all cases (Fig. 2b), except for *B. anomalus*, which poorly retained labelled DNA. Therefore, DNA sequences from this fragment could be useful for specific detection of *Dekkera* and *Brettanomyces* strains by PCR.

The 0.6-kb DNA fragment was sequenced, and the resulting sequence was compared to sequences available in current databases. This DNA fragment was chosen on the basis of its specific hybridization to *Dekkera* strain OSB101 DNA; however, as shown in Fig. 3a, this DNA sequence had significant homology with the *RAD4* gene from *S. cerevisiae*. To obtain a highly sensitive detection technique for *Dekkera* strains, we designed a two-step PCR method based on this sequence, but stretches of very low homology were selected to avoid false positives from *Saccharomyces* yeasts. By using this criterion, we designed two primers for the first reaction (DB1 [sense] and DB2 [antisense]) and two other nested primers for the second PCR (DB3 [sense] and DB4 [antisense]). The locations and orientations of these primers, as well as a schematic representation of the PCR procedure, are represented in Fig. 3. When the reaction was carried out with an excess of plasmid DNA containing the sequenced fragment, the first reaction yielded a PCR fragment 471 bp long, and the second yielded a fragment 327 bp long, as predicted from the target DNA sequence (Fig. 3).

To check the efficiency of the first PCR, samples containing the target DNA sequence in a plasmid were serially diluted and subjected to 30 cycles of amplification with primers DB1 and DB2. As shown in Fig. 4a, the target DNA produced an intense band of the expected size in ethidium bromide-stained agarose gels. According to the numbered serial dilution, a detection limit of 1,000 copies was achieved in this first PCR. A 1-μl sample of each PCR mixture was used to achieve a second

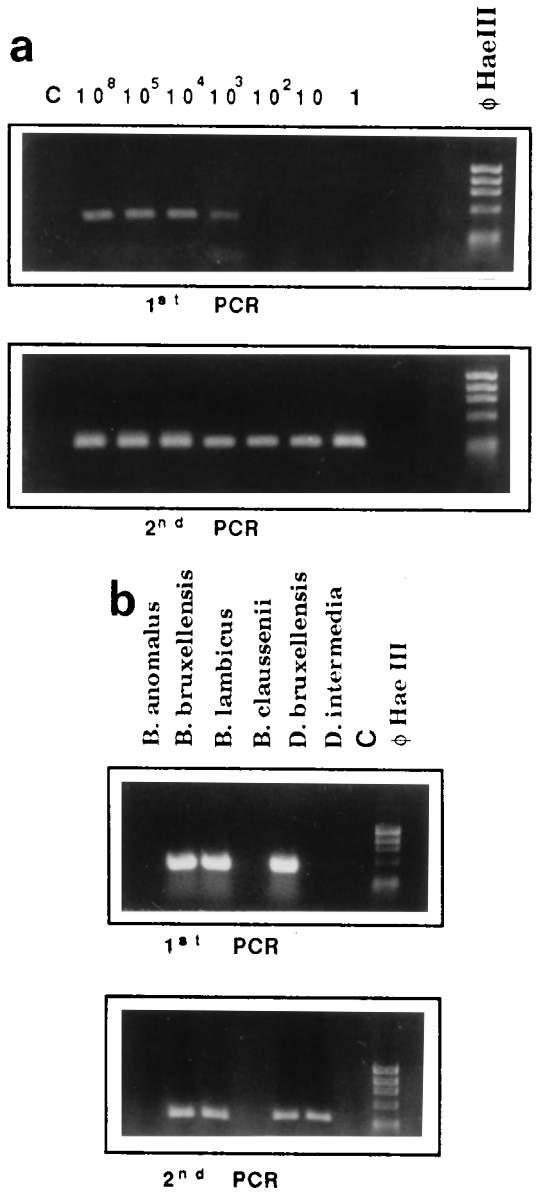


FIG. 4. (a) Sensitivity of the reaction. Shown is an ethidium bromide-stained agarose gel containing the PCR-amplified DNA obtained in the first and second nested PCRs by using serially diluted samples of plasmids containing the target DNA (values indicate the average numbers of plasmids loaded in the first PCR). The plasmid without a target DNA was used as a control (lane C). (b) Specificity of the PCR. Shown is an ethidium bromide-stained agarose gel containing the PCR-amplified DNA obtained in the first and the second PCRs by using DNAs from the indicated strains (DNA from *S. cerevisiae* MMY2 was used as a control [lane C]).

amplification with the DB3 and DB4 nested primers. After 30 cycles of amplification, up to a single copy of the plasmid in the original solution was detectable (Fig. 4a). To assay the detection of genomic sequences, DNA samples extracted from a number of yeast species were used in the nested PCR procedure; the reaction was performed as described in Materials and Methods for the amplification of plasmid DNA. The amplification rendered the expected products from *D. bruxellensis* OSB101, from all of the other *Dekkera* strains used (from *D. intermedia*, the expected DNA fragment was visualized only in the second PCR), and from *B. bruxellensis* and *B. lambicus*, but the reaction did not render observable DNA products when we used DNA from flor yeasts, from *B. anomalus*, or from *B. claussiensis* (Fig. 4b), indicating that the procedure is selective and specific for *D. bruxellensis* and synonymous strains (Table 1).

To simplify the procedure, we collected cells directly from a yeast colony (about 10^6 cells) and suspended the yeast cells in 100 μl of sterile water. A 10-μl volume of the cell suspension and subsequent dilutions were then used for the PCR. The nested PCR rendered possible the detection of fewer than 10 intact cells of *Dekkera* strains, with no need for DNA purification steps. We do not know how the PCR method works on intact cells; we have previously observed that removal of the cell wall is unnecessary for DNA molecules to move out of the cell by pulsed-field electrophoresis (10). Thus, denaturation of cell membranes and proteins during the heating steps of the PCR procedure might be enough to allow access of PCR reagents to the yeast DNA.

In sherry, *Saccharomyces* microorganisms are numerically superior; therefore, a practical method for early detection of *Dekkera* cells contaminating the wine requires specific and efficient amplification of a few *Dekkera* cells in samples containing a large number of *Saccharomyces* cells. To assay the interference that *Saccharomyces* cells may cause in the PCR, we prepared 1-ml suspensions of sterile water containing 10^8 *Saccharomyces* cells and increasing numbers of *Dekkera* strain OSB101 cells. Ten-microliter aliquots of these suspensions were used for a first round of 30 cycles of amplification (with primers DB1 and DB2), and 1 μl of the amplified product was used for the nested second-round reaction (with DB3 and DB4). As shown in Fig. 5a, the presence of a high density of *Saccharomyces* cells did not interfere with the specific amplification of *Dekkera* DNA from intact cells. Therefore, the nested PCR assay may be used as an efficient procedure for detection and identification of *Dekkera* strains by direct use of a cell suspension and with no interference from numerically superior *Saccharomyces* cells.

Monitoring of sherry. Wine is a chemically complex beverage that may also influence the enzymatic amplification of DNA. A sample of artificially contaminated sherry was prepared for direct PCR testing by the procedure described here. Ten-microliter aliquots of contaminated wine were directly used for PCR detection. Contrary to the previously described results (obtained by using cells suspended in water), only samples of wine containing more than 10^4 *Dekkera* cells per ml rendered positive nested PCR results. Similar results were obtained when purified DNA or a plasmid containing the target DNA was added to the wine, indicating that the sherry inhibited the PCR. To avoid this inhibitory effect, samples of contaminated wine were centrifuged, and the pellet was suspended in sterile water. PCRs with these samples showed sensitivity similar to that previously obtained with cells directly suspended in water. Thus, efficient and sensitive detection of *Dekkera* cells in sherry requires previous removal of the wine by centrifugation of the sample to be tested.

To assay the procedure in a practical case, we selected a barrel-aging sherry suspected of *Dekkera* contamination because of its high acetic acid content. Several 1.5-ml samples were collected from different locations in the barrel (from the wine and from the yeast film), the samples were centrifuged, and the pellet was suspended in 100 μl of sterile water. Ten-microliter aliquots of the suspension were used in the reaction. The nested PCR rendered the expected amplified DNA in all cases, indicating that *Dekkera* cells were present in the tested barrel (lanes 1 and 2 in Fig. 5b). Conventional culture methods verified the presence of the yeast 2 weeks later. Similar results

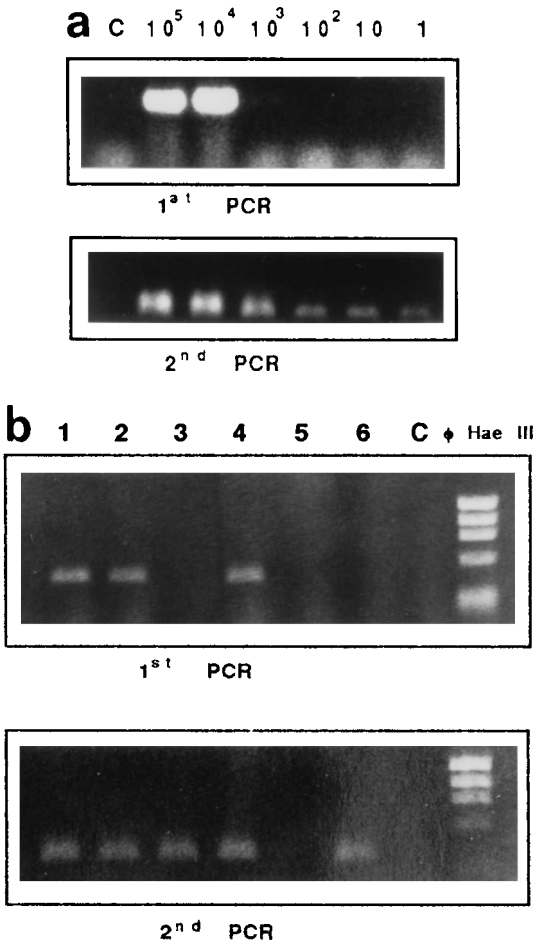


FIG. 5. Nested PCR amplification of DNA from intact cells. (a) Ethidium bromide-stained agarose gel containing the nested PCR-amplified DNA obtained by using the indicated number of intact *Dekkera* cells mixed with 10^6 *Saccharomyces* flor yeast cells (the C5 strain was used). Samples containing 10^6 *Saccharomyces* cells without *Dekkera* cells were used as a control (lane C). (b) Lanes 1 and 2 contained amplified DNA obtained from samples of a spoiled barrel suspected of contamination with *Dekkera* organisms. The samples were obtained from the sherry (lane 1) or from the yeast film (lane 2). Lanes 3 to 6 contained samples obtained from certain nonspoiled barrels either from the wine (lanes 3 and 5) or from the yeast film (lanes 4 and 6). Samples containing *Saccharomyces* flor yeast cells suspended in wine were used as a control (lane C).

were obtained with samples from certain nonspoiled barrels (lanes 3 to 6 in Fig. 5b) by means of this PCR procedure. The contaminating yeast was identified in the yeast layer growing on the surface, as well as in the wine, indicating that in the early stages of contamination, *Dekkera* cells may coexist with flor yeast cells in the surface.

In conclusion, we describe in this report the identification and genetic characterization of *Dekkera* yeasts contaminating sherry. The nested PCR developed in this work is excellently suited for detection and identification of this contaminating organism, with no need for isolation and culture. The total PCR assay can be performed in less than 10 h, simplifying current monitoring procedures. Nevertheless, sherry seems to inhibit the reaction, and efficient DNA amplification requires removal of the wine by centrifugation of the sample. Intact cells can be used, which obviates the tedious step of DNA purification. Thus, the method described here may be a reliable procedure for sensitive monitoring of *Dekkera-Brettanomyces* strains, which may help to prevent dissemination of these contaminant microorganisms in sherry and other beverages.

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