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REVIEW

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Yeast species recognition from gene sequence analyses and other molecular methods

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Abstract This review discusses DNA-based methods used for identification of yeasts. Nuclear DNA reassociation was the first quantitative molecular method employed for recognition of yeast species and has provided a baseline for interpretation of other molecular comparisons. Among these, gene sequencing is the most definitive method, with ribosomal RNA gene sequences providing the preponderance of available data. Multigene analyses that include the sequences of protein encoding genes are being increasingly developed to provide a more definitive resolution of species. A number of rapid identification methods, such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and flow cytometry, which are based on species-specific gene sequences, are available for use in diagnostic laboratories.

Key words Gene sequences · Molecular identification · Phylogenetic species · Rapid species identification · Yeasts

Introduction

Identification of yeast species from phenotypic characters, such as growth tests and cellular morphology, has been largely replaced by DNA-based methods. Various molecular studies, as well as genetic crosses, have shown that differences on fermentation and assimilation tests, as well as morphological differences such ascospore shape and presence or absence of hyphae and pseudohyphae, may vary among strains of a single species (Kurtzman and Phaff 1987; and references therein). As a consequence, DNA-based comparisons, especially of gene sequences, offer a "constant" upon which to base identification of species.

During the past 35–40 years, molecular identification of species has progressed from nuclear DNA reassociation to the sequencing of genes. In this review, various commonly used molecular taxonomic methods are discussed in an effort to understand their extent of genetic resolution and their application to rapid species identification.

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Whole genome comparisons from DNA reassociation and correlation with the biological species concept

The transition from phenotypic identification of yeasts to molecular identification began with determination of the mol% guanine + cytosine (G + C) ratios of nuclear DNA. These analyses demonstrated that ascomycetous yeasts range ~28–50 mol% G + C, whereas basidiomycetous yeasts range ~50–70 mol% G + C. Depending on the analytical methods used, strains differing by 1–2 mol% are recognized as separate species (Nakase and Komagata 1968; Price et al. 1978; Kurtzman and Phaff 1987). The need for quantitative assessment of genetic similarity between strains and species was satisfied, in part, by the technique of nuclear DNA reassociation or hybridization. DNA from the species pair of interest is sheared to a length of ~600 nucleotides, mixed, made single stranded, and the degree of relatedness between the pair is determined from the extent of reassociation. Many different methods are used to measure this process, which can be done spectrophotometrically or through use of radioisotopes or other markers (Kurtzman 1993).

Measurements of DNA complementarity are commonly expressed as percent relatedness. This usage can be misleading because DNA strands must show at least 75%–80% base sequence similarity before duplexing can occur and a reading is registered on the scale of percent relatedness (Bonner et al. 1973; Britten et al. 1974). Experimental conditions can greatly influence extent of duplex formation, but when measured under optimal conditions, different meth-

ods of assessing DNA relatedness give essentially the same result (Kurtzman 1993). Percent DNA relatedness provides an approximation of overall genome similarity between two organisms, but the technique does not detect single gene differences or exact multiples of ploidy, although aneuploidy can sometimes be detected (Vaughan-Martini and Kurtzman 1985).

A major question has been how to interpret DNA reassociation data. On the basis of shared phenotype, strains that showed 80% or greater nuclear DNA relatedness were believed to represent members of the same yeast species (Martini and Phaff 1973; Price et al. 1978). Correlation of DNA relatedness with the biological species concept has been examined from genetic crosses utilizing both heterothallic and homothallic species. In one of these studies, the heterothallic species *Pichia amylophila* Kurtzman et al. and *P. mississippiensis* Kurtzman et al., which showed 25% DNA relatedness, gave abundant interspecific mating, but ascus formation was limited and no ascospores were formed (Kurtzman et al. 1980a). Similar results were found for crosses between *Pichia americana* (Wick.) Kurtzman and *P. bimundalis* (Wick. & Santa Maria) Kurtzman (21% DNA relatedness) and between *Pichia alni* (Phaff et al.) Kurtzman and *P. canadensis* (Wick.) Kurtzman (*Hansenula wingei* Wick.), the latter pair showing just 6% DNA relatedness (Kurtzman 1984a,b). *Issatchenkia scutulata* (Phaff et al.) Kurtzman et al. and its variety *exigua* exhibited 25% DNA relatedness, but crosses between the two varieties gave mating and ascospore formation comparable to intravarietal crosses.

Ascospore viability from intervarietal crosses was ~5%, but sib-matings of the progeny had 17% ascospore viability. However, backcrosses to the parentals gave poor ascosporulation and very low viability (Kurtzman et al. 1980b), which suggests that these two varieties actually represent separate species. Smith et al. (2005) discovered four intermating populations of differing levels of DNA relatedness among isolates assigned to the heterothallic species *Zygoascus hellenicus* M.T. Sm. Two of the populations gave poor ascospore formation when intermated and showed only 25% DNA relatedness. These two populations were considered to be separate species, *Z. hellenicus* and *Z. meyerae* M.T. Sm. & V. Robert, the latter being newly described in the study. Strain matings within each of these two species gave a much higher frequency of ascospore formation than was observed for interspecific crosses. However, DNA reassociation resolved two populations within both species, each with 70% relatedness. Despite this reduced DNA relatedness, the two subpopulations within each species were considered to be conspecific because of the high frequency of ascospore formation, although ascospore viability was not determined.

Species of *Saccharomyces* are heterothallic and have been recognized from extent of nuclear DNA relatedness and/or multigene sequencing as well as from the biological species concept. One of the species, *S. pastorianus* E.C. Hansen, represents a hybrid between *S. cerevisiae* Meyen ex E.C. Hansen and *S. bayanus* Sacc. (Vaughan-Martini and Kurtzman 1985; Vaughan-Martini and Martini 1987; Yamada et al. 1993). Naumov et al. (2000) reported that with the exception of *S. pastorianus*, which appears sterile, the remaining six species of *Saccharomyces* can be crossed in any combination, but interspecific hybrids are sterile. With one exception, there is good correlation between species defined from DNA reassociation and from traditional genetic crosses. The exception is the species pair *S. cariocanus* G.I. Naumov et al. and *S. paradoxus* Bach., which gave 97% nuclear DNA relatedness when measured by spectrophotometic reassociation (Vaughan-Martini, personal communication). Other as yet unresolved species of *Saccharomyces* include American isolates of *S. paradoxus*, which give reduced fertility when crossed with European isolates (Naumov et al. 1998; Sniegowski et al. 2002), and *S. uvarum* Beij., which may be reproductively isolated from *S. bayanus* rather than being a synonym as proposed from their high nuclear DNA relatedness (Vaughan-Martini and Kurtzman 1985; Nguyen et al. 2000).

Studies of homothallic yeasts have given results similar to those from comparisons of heterothallic species. Although self-sporulating, cultures of homothallic species often show conjugation between independent cells and are therefore functioning as heterothallics. An example is a study of the five varieties of *Williopsis saturnus* (Klöcker) Zender in which auxotrophic markers were used to follow the mating process. The varieties of *W. saturnus* range in DNA relatedness from 37% to 79%. Intervarietal fertility was reduced and generally correlated with extent of DNA relatedness (Kurtzman 1987; and references therein). However, in some crosses, less-related pairs showed greater fertility than found among pairs with higher DNA relatedness. In other crosses, pairs with similar DNA relatedness showed somewhat different levels of fertility.

Conclusions are tentative from many of the genetic studies cited because they often did not involve a full test of the biological species concept. For a conclusive test, crosses must give meiotic spores with high viability that when crossed produce a highly fertile F_2 generation. Backcrosses to parentals should be highly fertile to rule out the possibility that the F_1 hybrids are allopolyploids (amphidiploids). Thus, a full test of the biological species concept can be quite time consuming, and when applied to homothallic species, reversion of auxotrophic markers can be a serious problem.

Despite the limitations discussed, the preceding studies show that mating among heterothallic as well as homothallic taxa can occur over a wide range of DNA relatedness values, but that highly fertile crosses, which demonstrate conspecificity, seem to exhibit 70% or greater DNA relatedness. Because species barriers are complex and involve a number of factors, the numerical range of 70%–100% DNA relatedness as indicative of conspecificity should be viewed as a prediction. This prediction has been applied to anamorphic as well as to teleomorphic species, because many anamorphs have been shown to represent mating types, and the genetic constraints affecting them may be essentially no different than those affecting teleomorphs.

Table 1. Correlation of nDNA relatedness and LSU D1/D2 divergence among ascomycetous yeasts[a]

Strain pairs	% nDNA relatedness	D1/D2 nucleotide differences
70 Conspecific pairs	70–100	0–3
~200 Unrelated pairs	0–20	6–250

[a] Data from Kurtzman and Robnett (1998, and references therein)

Gene sequence analyses

Nuclear DNA reassociation studies have had a marked impact on recognizing yeast species and are still useful, but the method is time consuming, and the extent of genetic resolution goes no further than that of closely related species. Nonetheless, this work has provided a frequently used baseline for interpretation of other types of molecular analyses.

Gene sequencing offers a rapid method for recognizing species and resolution is not limited to closely related taxa. Peterson and Kurtzman (1991) determined that domain 2 of large subunit (26S) ribosomal RNA (rRNA) was sufficiently variable to resolve individual biologically defined species. Kurtzman and Robnett (1998) expanded the preceding work by sequencing both domains 1 and 2 (D1/D2) (~600 nucleotides) of large subunit ribosomal DNA (rDNA) for all known ascomycetous yeasts, and Fell et al. (2000) published the D1/D2 sequences of known basidiomycetous yeasts, thus completing the database for all known yeasts. Consequently, this work has provided a universally available database for rapid identification of known species, the detection of new species, and initial phylogenetic placement of the species. Resolution provided by the D1/D2 domain was estimated from comparisons of taxa determined to be closely related from genetic crosses and from DNA reassociation. In general, strains of a species show no more than 0–3 nucleotide differences (0%–0.5%), and strains showing 6 or more noncontiguous substitutions (1%) are separate species (Table 1). Strains with an intermediate number of nucleotide substitutions are generally separate species as well. However, some exceptions to these predictions are discussed later. Use of the D1/D2 database has resulted in detection of a large number of new species, which has caused a near doubling of known species since publication of the most recent edition (4th) of *The Yeasts, A Taxonomic Study* (Kurtzman and Fell 1998). Another use for the D1/D2 database is that the nontaxonomist can now quickly and accurately identify most known species, as well as recognize new species, by sequencing ~600 nucleotides and doing a BLAST Search in GenBank.

The internal transcribed spacer (ITS) regions ITS1 and ITS2, which are separated by the 5.8S gene of rDNA, are also highly substituted and often used for species identification, but for many species ITS sequences give no greater resolution than that obtained from large subunit domains D1/D2 (James et al. 1996; Kurtzman and Robnett 2003). However, in contrast to this general observation, Kurtzman (2005) found ITS divergence useful for resolving closely related species in the ascomycetous genera *Saturnispora* and *Kregervanrija*. Furthermore, Fell and Blatt (1999) reported resolution of cryptic species in the *Xanthophyllomyces dendrorhous* Golubev species complex from ITS that had been unresolved by D1/D2 sequence analysis, and Scorzetti et al. (2002) reported ITS sequences to provide somewhat greater resolution among many basidiomycetous species than was found for D1/D2, although a few species were less well resolved by ITS than by D1/D2. In particular, Scorzetti et al. (2002) found no differences in ITS sequences between *Trichosporon laibachii* (Windish) E. Guého & M.T. Sm. and *T. multisporum* G. Cochet, but the pair had seven differences in the D1/D2 domain. Similarly, *Trichosporon montevideense* (L.A. Queiroz) E. Guého & M.T. Sm. had no ITS differences with *T. domesticum* Sugita et al., but there were two D1/D2 differences. Consequently, it appears useful to sequence both D1/D2 and ITS when comparing closely related species, and the value of additional gene comparisons cannot be overemphasized.

The intergenic spacer region (IGS) of rDNA tends to be highly substituted, and sequences of this region have been used with good success to separate closely related lineages of *Cryptococcus* (Fan et al. 1995; Diaz et al. 2000), *Xanthophyllomyces* (Fell and Blatt 1999), *Mrakia* (Diaz and Fell 2000), and *Saccharomyces* (Kurtzman et al., unpublished data). Because of the occurrence of repetitive sequences and homopolymeric regions, IGS tends to be difficult to sequence for some species. Resolution from small subunit (18S) rDNA, which has been extremely important in broad-based phylogenetic analyses, is generally not great enough to allow separation of many individual species (Peterson and Kurtzman 1991; James et al. 1996; Kurtzman and Robnett 2003).

The focus of our discussion on species identification from gene sequences has been on rDNA. A major advantage of rDNA is that it is present in all living organisms, has a common evolutionary origin, occurs as multiple copies, and is easy to sequence because primers pairs for the conserved regions that bracket the variable areas of interest can generally be used for all organisms. However, gene sequences other than those of the rDNA repeat have been used for separation of species from many kinds of fungi (Geiser et al. 1998; O'Donnell et al. 2000), including the yeasts. Belloch et al. (2000) demonstrated the utility of cytochrome oxidase II for resolution of *Kluyveromyces* species, Daniel et al. (2001) successfully used actin-1 for species of *Candida*, and Kurtzman and Robnett (2003) showed the usefulness of translation elongation factor 1-a and RNA polymerase II for resolution of *Saccharomyces* species. At present, the main impediment to widespread use of other gene sequences is developing sequencing primers that are effective for essentially all species and construction of databases that include sequences from all known species. Daniel et al. (2001) and Daniel and Meyer (2003) have made considerable progress in development of an actin sequence database for species identification, although no single primer pair has been effective for all known species, thus requiring additional primers to obtain these sequences. The need for

multiple primers is often a problem when sequencing protein encoding genes because nucleotide substitutions are often uniformly distributed, minimizing the likelihood of developing universal primers. Resolution of closely related taxa from actin sequences is somewhat greater than from D1/D2, but not surprisingly, clear separation of closely related species is not always certain (Daniel and Meyer 2003).

Resolution of species from single gene sequences can be affected by several factors. Among these, different lineages may vary in their rates of nucleotide substitution for the diagnostic gene being used, thus confusing interpretation of genetic separation, and hybrids are common and part of the speciation process. For example, Vaughan-Martini and Kurtzman (1985) proposed from DNA reassociation studies that *Saccharomyces pastorianus* is a natural hybrid of *S. cerevisiae* and *S. bayanus*, and Peterson and Kurtzman (1991) confirmed the proposal by showing that the D2 domain rRNA sequence of *S. pastorianus* is identical to that of *S. bayanus*, which is divergent from *S. cerevisiae*. The three varieties of *Candida shehatae* H.R. Buckley & Uden may also represent hybrids, or are examples of a lineage with a slower rate of nucleotide substitution in the rDNA. From DNA reassociation, the varieties show ~50% relatedness, but they have essentially identical domain 2 large subunit rRNA sequences (Kurtzman, 1990). Vaughan-Martini et al. (2005) presented a similar example from the *Pichia guilliermondii* Wick. clade. In their study, DNA reassociation resolved three closely related species: *P. guilliermondii*, *P. caribbica* Vaughan-Mart. et al., and *Candida carpophila* Vaughan-Mart. et al. The species range in DNA relatedness from 37% to 68%, but differ from one another by only 1–3 D1/D2 nucleotide substitutions (Table 2).

In other examples, Groth et al. (1999) discovered a natural chimeric isolate of *Saccharomyces* with genetic material from three species, and Nilsson-Tillgren et al. (1981) presented evidence that several natural and industrial yeast strains are hybrids. In an additional study, Lachance et al. (2003) found interfertile strains of *Clavispora lusitaniae* Rodr. Mir. that are highly polymorphic in the D1/D2 domain and which would appear to represent several different species if their identification were based solely on this particular gene sequence. It is often possible to detect unexpected divergence in a gene sequence from its lack of congruence with other gene sequences. Single gene sequences are extremely useful for rapid species identification, but from the foregoing examples, caution in interpretation of species identity is required.

Multigene identification of species and the phylogenetic species concept

Application of the biological species concept for recognition of all yeast species is not possible, because many species are known only from their anamorphic states, and for others, sexual states may be difficult to induce. It is for these reasons that nuclear DNA reassociation techniques became widely used among yeast taxonomists. With the development of rapid DNA sequencing technologies, it became evident that phylogenetic analysis of gene sequences could replace the slower, less-resolving DNA reassociation methods as a means for identifying species and understanding their genetic relationships.

Ribosomal RNA gene sequences became the obvious first choice for species comparisons for the reasons discussed earlier. What was not apparent in this early work was what biological units were being resolved from phylogenetic analysis. Did terminal lineages represent species, subspecies, or groups of species? Analysis of small subunit (18S) rDNA was shown to resolve groups of closely related species, whereas domains 1 and 2 of large subunit rDNA often resolved individual species (Peterson and Kurtzman 1991; James et al. 1996; Kurtzman and Robnett 1998). This interpretation was developed from comparisons of species recognized earlier from genetic crosses and from nuclear DNA reassociation. As already discussed, it has not been demonstrated that heterothallic, homothallic, and anamorphic species have markedly different rates of nucleotide substitution in D1/D2 or any other gene sequence, so the levels of resolution discussed in the preceding section have been tentatively applied to all three groups. Furthermore, correlation of D1/D2 rDNA divergence with results from genetic crosses and DNA reassociation showed that a phylogenetic species concept based on D1/D2 rDNA analysis generally parallels the biological species concept and

Table 2. Percent nuclear DNA relatedness and LSU D1/D2 nucleotide divergence among closely related species of the *Pichia guilliermondii* clade[a,b]

Species	Percent (%) nuclear DNA relatedness and D1/D2 nucleotide substitutions					
	P. guilliermondii		*P. caribbica*		*C. carpophila*	
	%DNA	D1/D2	%DNA	D1/D2	%DNA	D1/D2
Pichia guilliermondii	100	0	37	3	55	1
Pichia caribbica			100	0	68	2
Candida carpophila					100	0

[a] Data from Vaughan-Martini et al. (2005)
[b] DNA reassociation data are an average from five strain pairs of each species; all strains of each species had the same D1/D2 sequence

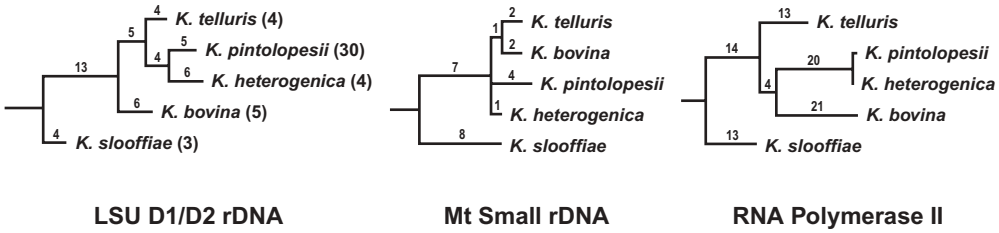

Fig. 1. Maximum-parsimony analyses of domains 1 and 2 large subunit (LSU) rDNA, mitochondrial small subunit rDNA, and RNA polymerase II gene sequences of *Kazachstania* species. The gene trees show overall congruence, but for RNA polymerase II, *K. pintolopesii* and *K. heterogenica* are nearly unresolved, suggesting that the latter species is a hybrid. The mitochondrial small subunit rDNA tree shows *K. telluris* and *K. bovina* to be more closely related than is indicated by the other two gene trees, again suggesting an interspecific hybridization event. *Numbers above branches* reflect nucleotide substitutions, and *numbers in parentheses* are the number of strains sequenced for each species

serves as a baseline for understanding resolution from other gene sequence analyses.

However, as seen from examples in the preceding section, the occurrence of hybrids and other genomic rearrangements can lead to incorrect identification of species, especially if identification is based on a single gene sequence. Multigene analyses offer a means for detecting these changes, which would be signaled by lack of congruence for a particular gene. This approach was recommended by Goodman (1976) for vertebrates, for bacteria by Dykhuizen and Green (1991), and for fungi by O'Donnell et al. (2000) and Taylor et al. (2000). The paper by Taylor et al. (2000) provides an inclusive review of species concepts.

Kurtzman and Robnett (2003) used multigene analyses to examine the population structure of species of *Saccharomyces*. Some strains of *S. cerevisiae*, *S. bayanus*, and *S. pastorianus* showed evidence of being hybrids. Multigene analyses of the *Kazachstania* (*Arxula*) *telluris* (van der Walt) Kurtzman species complex resolved five separate species (Kurtzman et al. 2005). In this work, it was shown that *K. heterogenica* Kurtzman & Robnett appears to be a natural hybrid that shares an RNA polymerase II gene with *K. pintolopesii* Kurtzman et al. (Fig. 1). Additionally, divergence between *K. telluris* and *K. bovina* Kurtzman & Robnett, based on mitochondrial small subunit rDNA, is less than expected, suggesting occasional gene exchange among members of the *K. telluris* species complex. Consequently, from the examples given, species can be most accurately identified from multigene sequencing using the phylogenetic species concept as the point of reference.

Rapid molecular methods for species identification and quantitation

Rapid molecular-based methods commonly used for species identification include species-specific primer pairs and probes, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), and karyotyping. Species-specific primers are effective when used for polymerase chain reaction (PCR)-based identifications involving a small number of species or when a particular species is the subject of the search (Fell 1993; Mannarelli and Kurtzman 1998; Chapman et al. 2003). Otherwise, there is the likelihood that PCR mixtures containing large numbers of species-specific primer pairs will lead to uncertain banding patterns. Microsatellite-primed RAPDs (Gadanho et al. 2003) and AFLP fingerprints (de Barros Lopes et al. 1999) have been effectively used in some laboratories. One concern in using these latter two techniques is reproducibility between laboratories because small differences in PCR conditions may impact the species-specific patterns that serve as reference. Karyotyping with pulsed-field electrophoresis and RAPD on mitochondrial DNA can serve in the initial characterization and identification of yeast species, but the interpretation of the chromosome band patterns and mitochondrial restriction fragments for taxonomic purposes is complicated by a high degree of polymorphisms, such as chromosomal rearrangements within some yeast taxa (Špírek et al. 2003).

High throughput probe hybridization methods are available for detection of multiple species in multiple samples. One method that appears effective for yeasts (Diaz and Fell 2004; Page and Kurtzman 2005) is an adaptation of the Luminex xMAP technology (Luminex Corp., Austin, TX, USA), which consists of a combination of 100 different sets of fluorescent beads covalently bound to species-specific DNA capture probes. Upon hybridization, the beads bearing the target amplicons are classified in a flow cytometer by their spectral addresses with a 635-nm laser. The hybridized biotinylated amplicon is quantitated by fluorescent detection with a 532-nm laser. The multiplex assay is specific and fast: species that differ by 1 nucleotide often can be discriminated and the assay can be performed, after amplification, in less than 50 min in a 96-well format with as many as 100 different species-specific probes per well. The advantage of this method for clinical, food quality, and ecology laboratories is that multiple species can be identified from multiple samples.

The preceding molecular detection methods are not designed to quantitate populations in various substrates; although the Luminex system has this capability, it has not yet been developed for yeasts. A number of factors affect detection and quantitation, including (1) cellular copy number of the gene to be used, (2) whether the gene is sufficiently conserved to be PCR amplified by "universal"

primers that will detect all species of interest, (3) efficiency of DNA extraction from cells in the sample, (4) efficiency of DNA recovery from the sample, (5) sample components that may interfere with DNA recovery or PCR amplification, and (6) level of cell population detectable.

Denaturing gradient gel electrophoresis (DGGE) is a promising technique that has been used for species identification and quantitation of yeast populations in foods and beverages. The technique is based on separation of DNA fragments of differing nucleotide sequences (e.g., species specific) through decreased electrophoretic mobility of partially melted double-stranded DNA amplicons in a polyacrylamide gel containing a linear gradient of DNA denaturants (a mixture of urea and formamide). A related technique is temperature gradient gel electrophoresis (TGGE), in which the gel gradient of DGGE is replaced by a temperature gradient (Muyzer and Smalla 1998). Recent applications of DGGE include identification and population dynamics of yeasts in sourdough bread (Meroth et al. 2003), in coffee fermentations (Masoud et al. 2004), and on wine grapes (Prakitchaiwattana et al. 2004). Levels of detection are often around 10^3 cfu ml^{-1}, but 10^2 cfu ml^{-1} have been reported, which compares favorably with standard plate count methods.

Prakitchaiwattana et al. (2004) provided information on mixed species populations, noting that when the ratio of species is not greater than 10 to 100 fold, detection of individual species was possible, but if the ratio exceeds 100 fold, the low population species will not be detected. Masoud et al. (2004) and Prakitchaiwattana et al. (2004) reported detection of species by DGGE that were not recovered by plating, suggesting that some yeasts may establish significant populations in a product and then die. Yet another rapid, quantitative molecular method is real-time PCR. This technique is becoming widely employed in food and beverage analyses and has been used to detect and quantitate spoilage yeasts in orange juice (Casey and Dobson 2004) as well as in wine fermentations (Cocolin et al. 2001).

Peptide nucleic acid (PNA) probes offer another means for detection and quantitation of species in clinical samples and food products through fluorescence in situ hybridization (FISH). PNA probes have a peptide backbone to which is attached nucleotides complementary to a species-specific target sequence, and a fluorescent label is added for detection by fluorescence microscopy (Stender et al. 2001). If probes are complementary to rRNA, the whole cell of the target species will "glow" when visualized, which will also allow quantitation by cell counts. An advantage is that a sample can be diluted and directly probed. One disadvantage is that probes must be developed for each species of interest, a problem common to most probe technology. PNA technology has been effective for detection of *Dekkera* (*Brettanomyces*) *bruxellensis* van der Walt in spoiled wine (Stender et al. 2001) and for detection of *Candida albicans* (C.P. Robin) Berkhout in blood samples (Rigby et al. 2002).

In summary, rapid detection, accurate identification, and quantitation of yeasts is now possible through use of a variety of molecular methods. Increased application of these methods will bring a greater degree of clarity to all questions in yeast microbiology, which previously was not possible when yeasts were identified from phenotype.

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