Chemotaxonomy of yeasts

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The present review deals with chemotaxonomic methods for yeasts. DNA base composition, which is expressed as molar percentages of guanine plus cytosine (G+C), is fitted for description of a new species and serves exclusionary functions. G+C content range among species within a genus is often 10% or less. Larger ranges may indicate an amalgam of genera. Typing and mapping of DNA may also be used for taxonomy. Strains showing 65% or greater relatedness after DNA-DNA hybridization may be considered members of the same species. With rRNA-DNA homology assessment, intrageneric relationships established are not usually meaningful, but intergeneric distances can be resolved. rRNA can be used for examining phylogenetic diversity of yeasts and alloenzyme variation to calculate genetic distances among large yeast populations. Furthermore, heterogeneity in coenzyme Q pattern, cytochrome spectra, composition of cell wall glucan, mannan, and chitin, and cellular fatty acids may serve chemotaxonomic purposes. □ *Fungi; chemistry; systematics*

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Yeasts can be described as unicellular fungi reproducing by budding or fission (1). They include a diverse group of organsims whose sexual stages are distributed between Ascomycotina and Basidiomycotina. Imperfect yeasts represent a third group with affinities both for ascomycetous and basidiomycetous yeasts. There are approximately 60 genera and 600 species of yeasts. Speciation originally relied on the morphology of vegetative and sexual stages and later on biochemical and genetic tests. Recently, chemotaxonomy has become increasingly useful.

Chemotaxonomy

Chemotaxonomy is concerned with the elucidation of the chemical composition of microbial cells or their products (2). Analysis of fermentation products and enzyme systems and their regulation may also be included in efforts made to demonstrate compounds that are specific, characteristic of, or unique for a particular group of organisms. Advances in analytic equipment related to chromatography and electrophoresis and to other physical and chemical techniques have led to major improvements in chemotaxonomy over the past decades. Research in chemical composition and nuclear deoxyribonucleic acid (DNA) base composition of yeasts has added characteristics to the standard descriptions of fundamental importance. Such characteristics are not routinely analyzed, but their assessment is the work of specialists. It is to be hoped that chemotaxonomy of yeasts will expand to a level at which it can be so easy to perform that it replaces/supplements traditional tests in the characterization of species and genera. The present review will give examples in which chemical methods have proved useful in yeast taxonomy.

DNA base composition

Several methods can be used to determine the base composition of nuclear or mitochondrial DNA in yeasts, expressed as the molar percentages of guanine plus cytosine (G+C) (1). DNA composition values are mainly exclusionary. This is not surprising, considering the fact that the approximately 600 yeast species known range from 27 to

70 mol% in G+C content, and overlap between unrelated species is inevitable. The fact that organisms have similar G+C contents does not necessarily mean that they have similar base sequences of their DNA.

The G+C content of ascomycetous yeasts is about 27–50%, whereas that of basidiomycetous yeasts varies between 50% and 70% (1). The taxonomic affinity of imperfect yeasts can also usually be determined from base composition, their G+C content varying from 35.5% to 67%.

Determination of base composition is particularly valuable in the description of new yeast species. The G+C content among species within a genus often varies within 10%. Differences much above 10% may suggest that the genus in which the organisms have been placed is composed of several genera.

Typing and mapping of DNA

DNA typing methods, based on digestion and electrophoresis of DNA fragments, appear to offer important potential advantages over phenotypic methods in taxonomy (3). Such methods provide a base for development of cloned probes for studies on DNA homology.

Restriction fragment length polymorphisms in the ribosomal DNA (rDNA) have also been used as a distinguishing characteristic to facilitate classification of clinically important yeasts. Magee et al. (4) found, in a sample of 12 clinical isolates of *Candida albicans*, 6 different classes based on variations in the fragments produced from genomic DNA by *Eco*RI and visualized after Southern transfer by being probed with a plasmid containing *Saccharomyces cerevisiae* rDNA. Similar digestion of other *Candida* species showed that each could be identified on the basis of its restriction enzyme pattern. Laaser et al. (5), using rDNA restriction fragment analysis as a taxonomic tool in separating physiologically similar basidiomycetous yeasts, found rDNA restriction analysis useful in establishing general taxonomic relationships, whereas DNA-DNA hybridization could only be used to exclude conspecificity. Six yeast species from the *Dekkera/Brettanomyces* genus, separated on the basis of physiologic characteristics, were grouped into three pairs after restriction endonuclease digestion of their mitochondrial DNAs (6). Yeast classification has also been attempted by mapping mitochondrial DNA (7). Recently, Merz et al. (8) defined electrophoretic karyotypes of *C. albicans* and found differences in the electrophoretic pattern among isolates from 14 different patients.

Select strains of *C. albicans* switch reversibly and at extremely high frequency between a white and an opaque colony-forming phenotype. The orthogonal field gel electrophoresis and rDNA restriction fragment profiles of white and opaque phenotypes were indistinguishable, and a genetic marker introduced into a white strain was present in all its opaque derivatives (9). Recently, opaque colonies isolated in parallel or in sequence after very short periods of time showed differences in Southern hybridization pattern with the highly mobile, moderately repetitive DNA sequence Ca7, suggesting that opaque colonies were not genetically identical (for a review, see Ref. 10).

DNA-DNA homology

DNA-DNA complementarity experiments measure the fidelity of complementary base pairing of renaturated DNA strands from test pairs. Leth Bak & Stenderup (11) synthesized radioactive complementary RNA in vitro, with each test DNA serving as a template. The relatedness between taxa could then be assessed from hybridization of single-stranded DNA with the synthesized RNA. DNA re-association can also be studied with radioactive preparations or spectrophotometrically by measuring the kinetics of duplex formation. DNA-DNA homology experiments are most valuable for species delimitations (12). Strains showing 65% or greater DNA relatedness may be considered conspecific, although the lower limit is not well defined (1). Values less than 20% usually indicate a distant relationship

between the strains (13). Complementary figures ranging between 20% and 35% may be difficult to interpret. Low DNA-DNA homology correlates with the lack of interfertility among yeast strains, since progressively less DNA relatedness parallels decreasing mating competence and fertility (1). When mating and DNA complementarity are correlated among closely related heterothallic ascomycetous and basidiomycetous yeasts, resolution of DNA-DNA reassociation goes no further than to detection of sibling species.

Several examples can be given demonstrating the validity of DNA-DNA homology experiments over that of traditional taxonomic criteria (1). Evidence has accumulated that presence or absence of hyphal development is not necessarily a good trait for species differentiation (14) but that the degree of DNA homology can be as, for example, in *Torulopsis* and *Candida* species (15). Yeast strains exposed to antimycotic agents often display altered physiologic characters. DNA-DNA complementarity may be used to identify unequivocally such strains (16). Rough-spored species of *Saccharomyces*, which are currently classified as *Torulaspora* (12), showed 98% or greater DNA relatedness and could therefore be considered conspecific. These species have previously been separated by differences in sugar assimilation and fermentation tests. Similarly, Leth Bak & Stenderup (11), using nucleic acid re-association as a means to determine relatedness among *Candida* species of medical interest, found *C. albicans, C. stellatoidea*, and *C. claussenii* to be conspecific, while *C. tropicalis* showed little relatedness to these taxa.

Ribosomal RNA

Ribosomal RNA (rRNA)-DNA homology studies have been made with species of relatively few genera. The data obtained suggest that intrageneric relationships established with this method are usually not meaningful owing to the high degree of conservation of the DNA sequences coding for rRNA (17). Such studies are promising in demonstrating intergeneric distances and, accordingly, more distant relationships between yeast species. Since the portion (approximately 2%) of genomic DNA encoding for rRNA is highly conserved, it has been suggested that an evolutionary hierarchy of yeasts based on rRNA-DNA homologies can be created (1). Genera, families, and, possibly, orders of basidiomycetous yeasts have been defined by 25S rRNA homology and correlated phenotypic characteristics (18). Other studies used 5S rRNA sequences to investigate taxonomic relationships among basidiomycetes and fungi imperfecti (19, 20). The sequences of *Pneumocystis carinii* 16S-like rRNA recently showed this organism to be a member of the fungi rather than of protozoa (21).

Enzymes

The amino acid sequence of enzymes such as glutamine synthetase and superoxide dismutase determined with quantitative microcomplement fixation may help evaluate the relationship between yeasts (1).

Coenzyme Q in yeasts vary in accordance with chain length from Q-6 to Q-10. Usually, species of one genus have the same Q-system, but exceptions exist in *Pichia* and *Hansenula* (17). Moreover, the number of isoprene units in the side chains of coenzyme Q differs from 5 to 10 (Q-5 to Q-10) among yeast taxa (22, 23). Studies of the coenzyme system of asporogenous yeasts demonstrated the taxonomic relationships between imperfect yeasts and certain groups of perfect genera (17).

Alloenzyme electrophoresis is based on the idea that genetic diversity develops over time. This implies that genetically distant organisms show a major difference in their protein composition and that more closely related organisms show a high degree of similarity. Alloenzyme analysis is more sensitive for studying intraspecific variation among yeasts than is DNA-DNA sequence complementarity (24). Holzschu (24) examined the banding patterns in starch gels of 14 metabolic enzymes in 400 strains of various cactophilic *Pichia* species, and the genetic

distances (intraspecific and interspecific) among various populations were determined. Specific alleles could be used for identification of all *Pichia* species and their varieties. Yamazaki & Komagata (25) concluded from electrophoretic comparison of 7 enzymes in 108 strains of *Rhodotorula* and *Rhodosporidium* that this technique was useful in establishing taxonomic relationships among red yeasts. Urease testing is also of value in yeast taxonomy (26).

Cellular protein/polypeptide profiles

Lehman et al. (27), using polyacrylamide gel electrophoresis of cellular proteins and several enzymes, found a close relationship between *C. tropicalis* and *C. parapsilosis*. These species were distinct from *C. albicans*. *C. stellatoidea* could be separated into two types. Schecter (28) applied disc electrophoresis of soluble proteins to taxonomic studies of dermatophytes and species of *Candida*. Autoradiographic analysis of profiles of (^{35}S)methionine-labeled cellular proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that different *Candida* species and other yeast species produced distinct patterns (29).

Killer system of yeasts

Killer toxins of yeasts may be useful for differentiating strain types among the pathogenic species and for surveillance of nosocomial infections caused by yeasts (30). They may also determine the distribution of pathogenic yeast strains within different countries. Moreover, killer yeasts have been classified by their toxin interaction (31).

Cytochrome spectra

Low-temperature cytochrome absorption spectra may be efficient tools in yeast systematics. Using such spectra, Montrocher & Claisse (32, 33) obtained results that corroborated the affinities of the species in the 'pseudotropicalis', 'robusta', and 'glabrata' groups, as evaluated by other molecular approaches. The low relatedness observed between these clusters and their close relationship with perfect genera such as *Kluyveromyces* or *Saccharomyces* pointed to the heterogeneity of the genus *Candida*.

Polysaccharides

Capsular polysaccharides have been used in yeast systematics. The fact that certain *Hansenula* and *Pichia* species contained slimy phosphomannons suggested a common ancestry (34). Several species of *Rhodotorula* were transferred to *Cryptococcus* because of similarities in capsular polysaccharides (35), and *Lipomyces* species have been separated on the basis of the presence or absence of D-galactose in the capsule (36). Mutants may diverge from this system.

In addition to the capsule, polysaccharides of yeast cell walls have been used in taxonomic studies, particularly on the generic level (37–39). Gas chromatographic-mass spectrometric analysis of hydrolysates from intact whole cells of species from the genus *Candida* recognized three distinct groups on the basis of carbohydrate patterns, reaffirming the heterogeneity of this genus (39). Using pyrolysis-mass spectrometry, Windig & Haverkamp (40) could distinguish between *Rhodosporidium* strains from their cellular hexose and *N*-acetylamino-sugar contents. The species *Saccharomyces cerevisiae*, *Rhodosporidium toruloides*, and *Filobasidium capsuligenum* could be differentiated on the basis of *N*-acetylamino-sugar, hexose, pentose, and protein composition (41). Differentiation between yeasts has also been made from absence or presence of glucuronic acid or D-xylose in the cell wall (37, 42).

Taxonomic information has also been gathered from the structural composition of yeast wall polysaccharides. Ascomycetous budding yeasts contain an alkali-insoluble, acid-insoluble β-(1-3)-glucan as the major structural component (42). Associated with this compound is an alkali-insoluble, acid-

soluble β-(1-6) glucan and an alkali-soluble β-(1-3) glucan with a large number of β-(1-6) bonds in its branched structure. The residual major component is a mannan–protein complex. Important differences have been demonstrated in the side chain of the branched mannan molecule (43). Controlled acetolysis of yeast mannans yielded mixtures of oligosaccharides with (1–2) and (1–3) linkages between the mannose units, whereas the less stable (1–6) linkages of the polysaccharide backbone were cleaved (44). The fingerprints obtained by gel filtration of the oligosaccharide mixtures, indicating proportion and size of side chains in various mannans, could be used to distinguish among different yeasts species. Proton magnetic resonance (PMR) spectra also provided sensitive criteria for distinguishing between mannans of different yeast species (45). Species with similar spectra probably had a close phylogenetic relationship. Another practical application of this technique has been the confirmation or rejection of postulated imperfect and perfect forms of the same yeast species (46) and tentative grouping of seemingly related species in heterogeneous asporogenous genera such as *Candida* (47). PMR spectra have also been used for confirmation of proposed phylogenetic lines of *Hansenula* and *Pichia* species (48). PMR spectra of mannans are not highly stable in cases of mutational modification of the cell envelope (1).

Chitin composition has been used to distinguish among yeasts. Whereas budding ascomycetous yeasts contain only a small amount of chitin (1–2% of the cell wall dry weight), the wall content in filamentous ascomycetous yeasts is considerably higher (42). Fission yeasts differ in several respects in cell wall composition from that of budding ascomycetous yeasts. The former lack chitin and contain α-(1-3)-glucan (49, 50), and their mannan has single galactose units attached to the backbone instead of mannose chain residues (43). Because of their unique cell wall composition, fission yeasts are not closely related to other ascomycetous yeasts. Cell walls of basidiomycetous yeasts usually contain β-glucan and chitin as the most important structural components (51).

Fatty acids

Total lipid content and examination of various classes of yeast lipids have been claimed not to offer much in taxonomy (52). However, cellular fatty acids were recently used to distinguish among *Candida albicans*, *Torulopsis glabrata*, and *Saccharomyces cerevisiae* after alcoholysis of whole cells (53), analytical data being subjected to multivariate statistical analyses. Kobayashi et al. (54) also suggested that the *Candida* genus can be distinguished from *Torulopsis* and *Saccharomyces* by gas–liquid chromatographic analyses of fatty acids. Further, long-chain fatty acids have been found to be valuable tools in the identification and classification of yeasts (55–57) and for studying the relationship between the perfect and imperfect stages of the genus *Candida* (58).

Conclusion

Chemotaxonomic methods should be increasingly used in classification and identification of yeasts. A major reason for this is that they provide exact, reproducible, and objective criteria for yeast systematics.

Conventional tests should be considered convenient and practical procedures of the routine diagnostic laboratory for separating taxa rather than basic indicators for yeast taxonomy.

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