Deoxyribonucleic Acid Relatedness among Species of the Genus Saccharomyces Sensu Stricto

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Twenty-four species assigned to the genus Saccharomyces sensu stricto were examined for deoxyribonucleic acid relatedness. Results with type strains demonstrated the presence of four distinct species: S. cerevisiae, S. bayanus, S. carlsbergensis, and S. kluyveri. S. carlsbergensis NRRL Y-126931 showed intermediate relatedness between S. cerevisiae and S. bayanus and has a genome size approximately 1.5 times those of the last two species. These data suggest S. carlsbergensis to be a partial amphidiploid which may have arisen from natural hybridization between S. cerevisiae and S. bayanus.

Numerous studies in recent years have shown that the criteria used in traditional yeast taxonomy are not always satisfactory for delimitation of species. This is partly because many separations are based on the presence or absence of assimilative abilities which are often controlled by a single mutable gene (12, 14, 18, 23, 25). The species of Saccharomyces sensu stricto (34) provide a good example of this problem because of strain variability in standard carbon assimilation and fermentation tests (5, 16, 28, 34, 35, 36). As a result of the uncertainty of these tests, a number of other approaches have been attempted, such as comparisons of cell wall antigens (2, 10, 11, 27), proton magnetic resonance spectra of extracted mannans (11, 32), and attempted matings between haploid mating types of similar species (3, 21). Requirements for vitamins (9) and amino compounds (7) have also been used for taxonomic separations, as have differences in deoxyribonucleic acid (DNA) base composition (guanine plus cytosine [G + C] contents in moles percent) (36). Finally, numerical analyses (1, 8, 15, 17) by Adansonian principles have been used for species separations. None of these methods, however, seems to have resolved the status of the species investigated (25).

More significant information concerning taxonomic relationships among yeasts is obtainable by comparison of their nucleic acids (18, 25). In this light, some progress has been made with regard to Saccharomyces spp. Bicknell and Douglas (6) determined DNA relatedness among several species, whereas Rosini et al. (26) concluded from their DNA studies that common wine yeasts comprised at least three Saccharomyces species. In this present study, we measured DNA relatedness among all taxa assigned to Saccharomyces sensu stricto (34, 35, 36) in an effort to clarify their relationships.

MATERIALS AND METHODS

Microorganisms. Twenty-nine strains representing 24 species were examined. Strain designations are given in Table 1.

DNA purification, determination of G + C contents, and reannealing reactions. Extraction and purification of DNA was accomplished by a combination of the procedures of Marmur (20) and Bernardi et al. (4), as described by Price et al. (25). Ratios of absorbance at 260:280 and 230:260 nm were used to assess DNA purity, as were thermal melting profiles and ultracentrifuge scans.

The G + C contents of nuclear DNAs were calculated from buoyant density values in cesium chloride (29, 33) based on three or four separate determinations made with a model E analytical ultracentrifuge (Beckman Instruments, Inc.) equipped with an electronic scanner. Micrococcus luteus (synonym, M. lysodeikticus) DNA was used as a reference; this DNA has a buoyant density of 1.7311 g/ml (25).

The extent of DNA reassociation was determined spectrophotometrically by using essentially the method reported by Seidler and Mandel (31) and Seidler et al. (30), as described by Kurtzman et al. (19).

Genome size. Estimates of genome size were determined by comparisons of C0.5 values (C0.5 = initial nucleotide concentration in moles per liter × time in seconds) obtained from reassociation kinetics (31). The relative genome size of S. cerevisiae was defined as 1.00 for this comparison.

Single-cell isolates. Single vegetative cell isolates, used in certain comparisons, were obtained by micromanipulation.

RESULTS

Origins of the strains and G + C contents of the nuclear DNAs are listed in Table 1. Neither habitat nor DNA base composition provided evidence of taxonomic value. Consequently, more significant comparisons were sought from DNA reassociation studies.

Results obtained from DNA hybridization studies (Tables 2 and 3) suggest that the 24 taxa compared comprise four species. Of these taxa, 15 showed high relatedness with S. cerevisiae (Table 2), and 5 showed high relatedness with S. bayanus (Table 3). Little relatedness was detected between S. kluyveri and the other species included in this study.

Of particular interest were reassociations involving S. carlsbergensis. This species and S. cerevisiae showed 57% relatedness, whereas the extent of its complementarity with S. bayanus was 72%. To preclude the possibility that mixed-stock cultures were not responsible for these results, we made single-cell isolates from the type strains of all three species. Reassociations involving DNA from single-cell isolates gave the same results. Relative genome sizes were estimated from reassociation kinetics (31) and found to vary among the type strains of the three species: S. cerevisiae, 1.00; S. bayanus, 1.22; and S. carlsbergensis, 1.49.

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taxonomic criteria. This philosophy led to the description of
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colonies" prompted van der Walt (34) to place it in his
tree exudates) from the other species placed in
Saccharomyces
carlsbergensis. This hybrid is probably a partial amphidiploid because its
 genome size is not quite double that of the proposed parents.
Furthermore, the S. bayanus parent of this hybrid was
closely allied although distinct from S. cerevisiae. Lastly,
Barker and Miller (3) obtained only infertile hybrids in
mating studies between S. kluyveri and S. cerevisiae despite
physiological cross-reactions of their mating pheromones
observed later by McCullough and Herskowitz (21).

Although our data clearly show S. carlsbergensis and S.
ayanus to be separate taxa, it is not evident why popula-
tions so similar in morphology, physiology, and habitat
would have evolved into separate species. Further studies
are needed to examine this interesting example of sympathy.

It was unexpected to find that S. carlsbergensis Y-12693
showed relatively high DNA relatedness to both S. cerevi-
siae and S. bayanus. These observations can be explained
by the proportionally larger genome size of this strain of S.
carlsbergensis. We suggest that S. carlsbergensis Y-12693
may represent a natural hybrid between these two species. This
hybrid is probably a partial amphidiploid because its
genome size is not quite double that of the proposed parents.

Furthermore, the S. bayanus parent of this hybrid was probably a strain formerly classified as S. uvarum because both S. carlsbergensis and S. uvarum ferment melibiose, whereas strains originally classified as S. bayanus did not

TABLE 1. Species of Saccharomyces sensu stricto examined

| Organism                      | Strain designation | Isolation source | G + C (moles percent) ± SD
|------------------------------|--------------------|------------------|------------------------
| S. abuliensis Santa Maria 1978| Y-11845            | Mesophylyx adoporus (Tr.) | 41.1 ± 0.24
| S. aceti Santa Maria 1959    | Y-12677            | Flor              | 41.8 ± 0.09
| S. bayanus Saccardo 1895     | Y-12624            | Beer              | 41.1 ± 0.24
| S. beticus Marcilla ex Santa Maria 1970 | Y-12625 | Single-cell isolate from Y-12624 | 41.1 ± 0.03
| S. capensis van der Walt et Tschueschner 1956 | YB-4237 | Grape must | 40.4 ± 0.13
| S. carlsbergensis Hansen 1908| Y-12629            | Grape must        | 40.4 ± 0.13
| S. cerevisiae Meyen ex Hansen 1883 | Y-12632          | Beer (bottom yeast) | 40.4 ± 0.13
| S. cerevisiae Meyen ex Hansen 1883 | Y-12632-1        | Single-cell isolate from Y-12632 | 40.4 ± 0.13
| S. chevalieri Guilliermond 1914 | Y-2034            | Wine              | 40.3 ± 0.14
| S. cordubensis Santa Maria 1970 | Y-12633            | Palm wine         | 40.3 ± 0.14
| S. coreanus Saito 1910        | Y-12636            | Wine              | 40.3 ± 0.14
| S. diastaticus Andrews et Gilliland ex van der Walt 1965 | Y-2416 | Grape must | 40.2 ± 0.04
| S. goditensis Santa Maria 1970| Y-12644            | Highly infected gravity | 40.3 ± 0.14
| S. globosus Osterwalder 1924  | Y-12645            | Wort              | 40.2 ± 0.04
| S. heterogenicus Osterwalder 1924 | Y-12646          | Beer              | 41.6 ± 0.06
| S. hieniensis Santa Maria 1962| Y-6677             | Fruit must        | 40.1 ± 0.17
| S. hispalensis Santa Maria 1978| Y-11846            | ‘Alpechin’        | 40.5 ± 0.12
| S. itaticus van der Walt 1965 | Y-12648            | ‘Alpechin’        | 40.5 ± 0.12
| S. kluyveri Phaff, Miller, et Shifrine 1956 | Y-12649 | Grape must | 40.1 ± 0.17
| S. norborensis Santa Maria 1963| Y-12651            | Drosophilina pinicola | 41.0 ± 0.13
| S. oleaceus Santa Maria 1958  | Y-12657            | ‘Alpechin’ | 41.0 ± 0.13
| S. oleaginosus Santa Maria 1958| Y-6679             | ‘Alpechin’    | 41.0 ± 0.13
| S. prostoserdovii Kudriavzve 1960 | Y-12659        | Feces              | 40.1 ± 0.17
| S. uvarum Beijerinck 1898    | Y-12660            | Grape must        | 40.1 ± 0.17
| S. uvarum Beijerinck 1898    | Y-12663            | Currant juice     | 42.1 ± 0.11

* Mean ± standard deviation. Standard deviations were calculated from three determinations.

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DISCUSSION

The controversies that have surrounded definition of spe-
cies in Saccharomyces sensu stricto were caused, for the
most part, by heavy reliance on fermentation and assimila-
tion of carbon compounds, particularly sugars, as primary
taxonomic criteria. This philosophy led to the description of
many taxa that we have now shown to be synonyms of S.
cerevisiae or S. bayanus. Although some conspecificity
within Saccharomyces sensu stricto has been recognized in
recent years after reevaluation of data from standard mor-
phological and physiological tests (5, 35, 36), these tests
appear incapable of discriminating all species. For example,
S. carlsbergensis has been considered a synonym of S.
uvarum (34) as well as of S. cerevisiae (35).

Confirmation of S. kluyveri as a distinct species was
expected for a number of reasons. Its ecological isolation (in
tree exudates) from the other species placed in Saccharo-
ymces sensu stricto, as well as its inability to form ‘‘petite
colonies’’ prompted van der Walt (34) to place it in his
Saccharomyces group II. Meyer and Phaff (22) and Yarrow
and Nakase (36) transferred it to group I; they considered it
ferment this sugar. Bicknell and Douglas (6) reported 96% DNA homology between *S. cerevisiae* and *S. carlsbergensis* and 40% homology between *S. cerevisiae* and *S. uvarum* (*S. bayanus*). The reason for the difference between their results and ours is unknown. We can only comment that their cultures were not the type strains of these species. In support of the idea that *S. cerevisiae* and *S. carlsbergensis* are separate taxa, Holmberg (13) noted that these two species had distinct differences in various zones of chromosome III. Additionally, Nilsson-Tillgren et al. (24) have demonstrated the possibility of single-chromosome transfers between *S. cerevisiae* and *S. carlsbergensis*. In the absence of contrary genetic evidence, we might expect that *S. carlsbergensis* would not form fully fertile hybrids with either *S. cerevisiae* or *S. bayanus*. If this prediction proves true, *S. carlsbergensis* will have to be considered a species distinct from its parents.

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