Deoxyribonucleic Acid Base Sequence Relatedness Among Strains of *Pichia ohmeri* That Produce Dimorphic Ascospores

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Strains of *Pichia ohmeri* that form either spherical or hat-shaped ascospores comprise a well-defined assemblage, distinct in deoxyribonucleic acid base sequence and composition from other phenotypically similar yeasts. Thus, spore morphology, usually a stable systematic character, is not invariably reliable as a major taxonomic criterion among yeasts.

Ascospore shape is normally considered a cardinal taxonomic character among yeasts and in some instances is the sole criterion for the separation of species or even genera (4). Since the genetics of spore shape has not yet been established, however, it is possible that striking differences in morphology could depend on only one or a few genes. If this is the case, ascospore shape could prove a less reliable character than heretofore imagined. Consistent with this notion, spore shape in most yeasts appears constant and species specific, but the exceptions cannot be ignored. In both *Endomycopsis lipolytica* (11) and *Pichia membranaefaciens* (3, Kurtzman, personal communication), a single strain may produce distinctly different ascospores.

In *Pichia ohmeri*, Wickerham and Burton (10) observed an unusual case: crosses between different pairs of haploid strains produced either spheroidal or hat-shaped spores. These authors did not establish the fertility of the hybrid ascospores, however, and thus some question remained whether the various strains represent a single genospecies or whether the uncommon mating behavior reflects residual relatedness among divergent yeasts. We have, therefore, used deoxyribonucleic acid (DNA) sequence comparison to assess the overall genetic similarity among several *P. ohmeri* strains of complementary mating type as well as their kinship to other phenotypically similar *Pichia* species.

MATERIALS AND METHODS

Organisms. The yeasts used in this study are shown in Table 1.

DNA purification. Cultivation of cells and the extraction and purification of whole-cell yeast DNA have been described elsewhere (7).

Determination of base composition. The guanine + cytosine (G+C) content of yeast nuclear DNA

was calculated from the buoyant density in cesium chloride by using the equation of Schildkraut et al. (8), as previously described (7).

DNA reannealing reactions. Sequence complementarity was determined by renaturation in 280 mM PB (an equimolar mixture of mono- and dibasic sodium phosphate, pH 6.8) at 25°C below the T_m of native homologous DNA, as described in detail earlier (7). Rapidly renaturing minor DNA species were removed from the ³²P-labeled reference DNA before reassociation (7). After reannealing reactions, duplex molecules were separated from unreacted DNA with 140 mM PB at 60°C by the hydroxylapatite batch technique (1). A PB molarity of 90 mM was used for thermal elution studies of duplex molecules (5, 7). The data are presented as percentage of actual binding to hydroxylapatite (experimentally determined) and as percentage of relative binding (normalized to the homologous reaction).

RESULTS AND DISCUSSION

The yeast strains used are listed in Table 1. Among the strains of *P. ohmeri*, 75-54 (h⁻) produces only round spores when crossed with strains of opposite mating type (e.g., 75-52, h⁺), whereas 75-53 (h⁻) produces exclusively hat-shaped spores in crosses with 75-52 (h⁺) (10). An exceptional case is strain NRRL Y-1260, which yields hat-shaped ascospores when mated with either 75-53 or 75-54. The type strain 75-74 was isolated as a diploid, but became an asporogenous haploid during laboratory cultivation. Its mating type with respect to the other strains was not determined. We also compared strains of several *Pichia* species that either have phenotypic resemblance to *P. ohmeri* (3) or are similar in DNA base composition (6).

Our base composition data (Table 2) show that all four strains of *P. ohmeri*, the type strain of *P. guilliermondii*, and the type strain of *P. membranaefaciens* are indistinguishable on this basis. *P. pinus* and *P. fermentans* are significantly different from *P. ohmeri* in base composition. The phenotypic properties of these two species also differ considerably from those of *P.*

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ohmeri, and the natural habitat of *P. pinus* is distinct from that of *P. ohmeri* (3). None of the other yeasts studied is likely to be closely related to *P. ohmeri*, since in our experience yeasts with

TABLE 1. Yeast strains employed

Species name	Strain designation ^a		
	UCD	CBS	NRRL
<i>Pichia fermentans</i> Lodder	56-5 ^b	187	Y-1619
<i>P. guilliermondii</i> Wick-erham	75-74 ^b	2030	Y-2076
<i>P. membranaefaciens</i> Hansen	57-22 ^b	107	Y-2026
<i>P. ohmeri</i> (Etchells et Bell) Kreger-van Rij	75-73 ^b	5367	Y-1932
	75-52	2037	Y-2078
	75-53	568	Y-2079
	75-54	2038	Y-2080
<i>P. pijperi</i> van der Walt et Tscheuschner	61-19 ^b	2887	
<i>P. pinus</i> (Holst) Phaff	75-76 ^b	744	
<i>P. rhodanensis</i> (Ramirez et Boidin) Phaff	56-7 ^b	5518	Y-7854
<i>P. scolyti</i> (Phaff et Yoneyama) Kreger-van Rij	59-62 ^b	4802	
<i>P. strasburgensis</i> (Ramirez et Boidin) Phaff	56-8 ^b	2939	Y-2383
<i>P. wickerhamii</i> (van der Walt) Kreger-van Rij	60-23 ^b	4107	

^a Culture collection abbreviations: UCD, Department of Food Science and Technology Culture Collection, University of California, Davis; CBS, Centraal Bureau voor Schimmelcultures, Delft, Netherlands; NRRL, Northern Regional Research Laboratory, Peoria, Ill.

^b Type strain.

DNA base compositions differing by more than 1 to 2 mol% G+C (as determined from the buoyant density) do not have significant DNA complementarity (2, 7; Phaff, Presley, and Holzschu, unpublished data). DNA base sequence comparisons (Table 3) show that all four strains of *P. ohmeri* have high sequence relatedness and little in common with the other species tested. Thus, the *P. ohmeri* strains form a well-defined assemblage genetically distinct from all other phenetically similar yeasts.

TABLE 2. Base composition of nuclear DNA of 13 yeasts of the genus *Pichia*

Species	Strain designation ^a	Mol% G+C ^b ± SD
<i>Pichia scolyti</i> ^c	59-62	40.1 ± 0.26
<i>P. strasburgensis</i> ^c	56-8	40.6 ± 0.23
<i>P. pijperi</i> ^c	61-19	42.5 ± 0.09
<i>P. pinus</i> ^c	75-76	43.0 ± 0.06
<i>P. fermentans</i> ^c	56-5	43.1 ± 0.27
<i>P. membranaefaciens</i> ^c	57-22	44.3 ± 0.44
<i>P. ohmeri</i> ^c	75-73	44.3 ± 0.19
<i>P. guilliermondii</i> ^c	75-74	44.4 ± 0.10
<i>P. ohmeri</i>	75-54	44.5 ± 0.21
<i>P. ohmeri</i>	75-53	44.6 ± 0.47
<i>P. ohmeri</i>	75-52	44.7 ± 0.24
<i>P. wickerhamii</i> ^c	60-23	45.6 ± 0.26
<i>P. rhodanensis</i> ^c	56-7	51.9 ± 0.15

^a University of California at Davis, Food Science and Technology Collection strain number.

^b Calculated from the average of at least four buoyant density determinations. Braces enclose those base composition values which are not separable at the 95% confidence level (Student-Newman-Keuls a posteriori range test [9]). SD, Standard deviation.

^c Type strain.

TABLE 3. Percent reannealing between ³²P-labeled DNA from *P. ohmeri* 75-52 and DNA from seven strains of yeasts^a

Organism or tissue	Strain designation ^b	% Actual binding ^c	% Relative binding ^d	ΔT _{m(e)} ^e
<i>P. ohmeri</i>	75-52	89.7 ± 1.18	(100)	
<i>P. ohmeri</i> ^f	75-73	89.6 ± 0.18	100.0	0.7
<i>P. ohmeri</i>	75-53	88.3 ± 0.48	98.4	0.6
<i>P. ohmeri</i>	75-54	88.0 ± 0.60	98.0	0.6
<i>P. guilliermondii</i> ^f	75-74	10.6 ± 1.21	6.8	— ^g
<i>P. membranaefaciens</i> ^f	57-22	6.7 ± 0.84	2.2	— ^g
<i>P. wickerhamii</i> ^f	60-23	6.6 ± 0.23	2.1	— ^g
Calf thymus		7.2 ± 0.89	2.8	— ^g

^a A 0.2-μg amount of ³²P-labeled DNA (ca. 75,000 cpm/μg) and 200 μg of unlabeled DNA in 0.5 ml of 280 mM PB at 65°C.

^b University of California at Davis, Food Science and Technology Collection strain number.

^c Average of triplicate samples corrected for zero-time binding = 0.12 ± 0.18%.

^d Corrected for self-renaturation of labeled DNA = 4.82 ± 0.77%.

^e T_{m(e)} is the thermal elution midpoint, the temperature at which 50% of the adsorbed DNA is eluted from the hydroxylapatite. ΔT_{m(e)} is the decrease in T_{m(e)} between the heterologous reaction and the homologous (*P. ohmeri* 75-52) reaction.

^f Type strain.

^g ΔT_{m(e)} is not meaningful because of the substantial contribution of self-reassociated, labeled DNA to the thermal elution profile of this heterologous reaction.

Although matings between the four *Pichia ohmeri* strains produce spores of different shapes, our data demonstrate their close kinship. Furthermore, Kurtzman (personal communication) has recently found that a few fertile progeny are produced by crosses among these same strains. Thus, *P. ohmeri* constitutes a valid species on both molecular and biological grounds. Our results indicate that differences in spore morphology do not necessarily reflect differences in the entire genomes of yeasts and should not be used as the basis for species separation unless supported by other criteria.

ACKNOWLEDGMENTS

This research was supported by Public Health Service Research grant GM-16307-08 from the National Institute of General Medical Sciences.

We gratefully acknowledge the capable assistance of Nancy Krauter in the DNA isolation and of Mary Miranda for diagnostic testing. We thank C. P. Kurtzman for supplying several of the yeast strains and for helpful discussions.

REPRINT REQUESTS

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