A Numerical Classification of Fission Yeasts of the Genus *Schizosaccharomyces* Lindner

By PAUL D. BRIDGE*† AND JOHN W. MAY

Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia

(Received 10 January 1984; revised 26 March 1984)

A numerical classification of the yeast genus *Schizosaccharomyces* was undertaken using 60 strains and 100 characters. Three distinct clusters were observed, corresponding to *S. pombe* ($S_{SM} = 83\%$), both varieties of *S. japonicus* ($S_{SM} = 78\%$), and *S. octosporus* ($S_{SM} = 75\%$). *Schizosaccharomyces malidevorans* and *S. slooffiae*, each of which is available only as the type, could not be differentiated from *S. pombe* and *S. octosporus* respectively.

INTRODUCTION

The yeast genus *Schizosaccharomyces* was first described by Lindner from samples of Bantu beer (Slooff, 1970). All members of the genus undergo binary fission during vegetative reproduction, a characteristic unique among ascosporogenous yeasts (Lodder, 1970; Barnett *et al.*, 1979). The history of the genus is well described by Slooff (1970) who listed four species including two varieties: *S. japonicus* var. *japonicus*, *S. japonicus* var. *versatilis*, *S. malidevorans*, *S. octosporus* and *S. pombe*, the last being the type species. Subsequently, a fifth species, *S. slooffiae*, was described by Kumbhojkar (1972); however, more recently, *S. malidevorans* was re-proposed as a variety of *S. pombe* on the basis of interfertility (Sipiczki *et al.*, 1982).

During the last three decades, the major influence in yeast classification has been *The Yeasts – a Taxonomic Study* (Lodder & Kreger-van Rij, 1952; Lodder, 1970). In contrast to the hierarchical approach employed in the foregoing work, the assessment of overall similarity on the basis of equal weighting of characters combined with the use of numerical processing procedures has also been employed with several genera of yeasts (Campbell, 1972, 1973, 1975; Kockova-Kratochvilova *et al.*, 1978). For such an assessment to be valid, at least 50 characters should be used in the comparisons (Sneath, 1978); however, of the 78 characters listed for yeasts by Barnett *et al.* (1979), only 15 were differential for fission yeasts. In this paper we describe a set of traditional and novel characters which we have used to construct a numerical classification of the genus *Schizosaccharomyces*.

METHODS

Media. The basic media were: MEA – Malt Extract Agar (Oxoid); PYGM – peptone, yeast extract, glucose medium (Conti & Naylor, 1959), EMM2A – Edinburgh minimal medium no. 2 (Mitchison, 1970) supplemented with adenine (50 mg l^{-1}); EMM2A(G-3) – EMM2A in which glucose, the sole carbon source, was reduced from 10 to 3 g l^{-1} , and EMM2A(G-0) – EMM2A from which the glucose was omitted. When required, 1% (w/v) agar (no. 1, Oxoid) was added to EMM2A or one of its variants.

Strains. The strains used are listed, in cluster order, in Table 1. Strains were checked for purity on receipt and maintained on slopes of MEA at 4°C .

Growth conditions. All cultures were incubated aerobically at 30°C without shaking unless otherwise stated.

Preparation of inocula. Each strain to be tested was grown in PYGM for 1 d. After harvesting by centrifugation, cells were washed once with and then resuspended in EMM2A(G-0) to give an absorbance reading of 0.1 at a wavelength of 550 nm in a spectrophotometer (Spectronic 20, Bausch & Lomb).

† Present address: Commonwealth Mycological Institute, Kew, Surrey TW9 3AF, UK.

Table 1. *Strains arranged in cluster order (S_{SM} coefficient)*

Monash designation	Name as received	Source* and designation	Comments*
MY1	<i>Schizosaccharomyces pombe</i> Lindner	AWRI 160	Vineyard
MY2	<i>S. pombe</i> Lindner	AWRI 161A	UCD 227
MY27	<i>S. pombe</i> Lindner (syn. <i>S. asporus</i> Beijerinck)	IFO 0340	
MY28	<i>S. pombe</i> Lindner (syn. <i>S. formosensis</i> Nakazawa var. <i>tapaniensis</i> Nakazawa)	IFO 0344	CBS 1062, NRRL Y-1358; cane-sugar molasses
MY3	<i>S. pombe</i> Lindner	AWRI 161	
MY35	<i>S. pombe</i> Lindner (syn. <i>S. vordermani</i> Wehmer)	IFO 0366	
MY4	<i>S. pombe</i> Lindner	L972	Obtained via JMM;
MY18	<i>S. pombe</i> Lindner (syn. <i>S. formosensis</i> Nakazawa var. <i>akoensis</i> Nakazawa)	CBS 1044	ATCC 24969, NCYC 1354 IFO 0343; cane-sugar molasses
MY6	<i>S. pombe</i> Lindner		Variant of MY5 (Mahadi, 1977)
MY15	<i>S. pombe</i> Lindner	CBS 356	Type strain; UCD C277
MY8	<i>S. malidevorans</i> Rankine & Fornachon	AWRI 158	Type strain; CBS 5557, IFO 1608, NCYC 683, RIVE 4-6-1, UCD 70-49; Listan grapes
MY16	<i>S. pombe</i> Lindner (syn. <i>S. liquefaciens</i> Osterwalder)	CBS 1042	ATCC 16979, IFO 0358; grape juice
MY19	<i>S. pombe</i> Lindner	CBS 1059	Cane sugar
MY9	<i>Schizosaccharomyces</i> sp.	RYCC 24/5	Sulphited grape juice
MY10	<i>Schizosaccharomyces</i> sp.	RYCC 24/6	Sulphited grape juice
MY11	<i>Schizosaccharomyces</i> sp.	RYCC 24/7	Sulphited grape juice
MY12	<i>Schizosaccharomyces</i> sp.	RYCC 24/4	Sulphited grape juice
MY17	<i>S. pombe</i> Lindner (syn. <i>S. formosensis</i> Nakazawa)	CBS 1043	IFO 0342; cane-sugar molasses
MY42	<i>S. mosquensis</i> Shcerbakov & Popova	RIVE 4-2-1	
MY47	<i>S. pombe</i> Lindner	CBS 5682	Bantu beer
MY48	<i>S. malidevorans</i> Rankine & Fornachon	CBS 5557	Type strain; AWRI 158, IFO 1608, NCYC 683, RIVE 4-6-1; UCD 70-49; Listan grapes
MY29	<i>S. pombe</i> Lindner (syn. <i>S. pinan</i> Nakazawa)	IFO 0345	CBS 355, NRRL Y-1359; molasses
MY33	<i>S. pombe</i> Lindner (syn. <i>S. santawensis</i> Nakazawa)	IFO 0364	CBS 1063, NRRL Y-1362; cane-sugar molasses
MY41	<i>S. mellacei</i> Jorgensen	RIVE 4-5-1	
MY30	<i>S. pombe</i> Lindner (syn. <i>S. pombe</i> Lindner var. <i>ogasawaraensis</i> Sakaguchi & Ohtani)	IFO 0347	CBS 2775
MY31	<i>S. pombe</i> Lindner (syn. <i>S. pombe</i> Lindner var. <i>iotoensis</i> Sakaguchi & Ohtani)	IFO 0349	CBS 2776
MY32	<i>S. pombe</i> Lindner (syn. <i>S. liquefaciens</i> Osterwalder)	IFO 0358	ATCC 16979, CBS 1042; grape juice
MY43	<i>S. acidodevoratus</i> Tschalenko	RIVE 4-1-1	
MY34	<i>S. pombe</i> Lindner (syn. <i>S. taito</i> Nakazawa)	IFO 0365	CBS 1061, NRRL Y-1605; cane-sugar molasses
MY44	<i>S. pombe</i> Lindner	CBS 357	Cane-sugar molasses
MY46	<i>S. pombe</i> Lindner	CBS 2777	IFO 0348
MY56	<i>S. pombe</i> Lindner	NCYC 132	ATCC 24751; millet beer
MY57	<i>S. pombe</i> Lindner	NCYC 936	Fermenting toddy
MY58	<i>S. pombe</i> Lindner	NCYC 380	Cane sugar
MY55	<i>S. pombe</i> Lindner	CBS 1058	Molasses
MY51	<i>S. pombe</i> Lindner	IFO 0346	
MY52	<i>S. pombe</i> Lindner	IFO 0351	
MY53	<i>S. pombe</i> Lindner (syn. <i>S. liquefaciens</i> Osterwalder)	IFO 0638	

Table 1 (*continued*)

Monash designation	Name as received	Source* and designation	Comments*
MY5	<i>S. pombe</i> Lindner	NCYC 132	Obtained via JMM; ATCC 24751; millet beer
MY45	<i>S. pombe</i> Lindner	CBS 2628	Palm wine
MY54	<i>S. pombe</i> Lindner	CBS 1057	From brewer's yeast
MY13	<i>S. japonicus</i> Yukawa & Maki var. <i>japonicus</i>	AWRI 156	Type strain: ATCC 10660, CBS 354, IFO 1609, NRRL Y-1361, UCD 71-26; strawberry wine
MY14	<i>S. japonicus</i> Yukawa & Maki var. <i>versatilis</i> (Wickerham & Duprat) Slooff	AWRI 157	Type strain; ATCC 9987, CBS 103, IFO 1607, NRRL Y-1026E; home- canned grape juice
MY21	<i>S. japonicus</i> Yukawa & Maki var. <i>versatilis</i> (Wickerham & Duprat) Slooff	CBS 5679	ATCC 24256, UCD 60-255; slime flux of <i>Ulmus</i> <i>carpinifolia</i>
MY36	<i>S. japonicus</i> Yukawa & Maki var. <i>japonicus</i>	IFO 1646	Slime flux of tree
MY38	<i>S. japonicus</i> Yukawa & Maki var. <i>japonicus</i>	IFO 1713	
MY61	<i>S. japonicus</i> Yukawa & Maki var. <i>versatilis</i> (Wickerham & Duprat) Slooff	NCYC 419	
MY20	<i>S. japonicus</i> Yukawa & Maki var. <i>versatilis</i> (Wickerham & Duprat) Slooff	CBS 2629	NRRL Y-2390; white wine
MY37	<i>S. japonicus</i> Yukawa & Maki var. <i>japonicus</i>	IFO 1712	
MY7	<i>S. octosporus</i> Beijerinck	AWRI 159	
MY25	<i>S. octosporus</i> Beijerinck	CBS 6209	Honey
MY23	<i>S. octosporus</i> Beijerinck	CBS 2632	
MY24	<i>S. octosporus</i> Beijerinck	CBS 6206	Honey
MY26	<i>S. slooffiae</i> Kumbhojkar	CBS 6207	Type strain; NRRL Y-7951; honey
MY39	<i>S. octosporus</i> Beijerinck	IFO 0353	
MY40	<i>S. octosporus</i> Beijerinck	IFO 0360	
MY60	<i>S. octosporus</i> Beijerinck	NCYC 427	Cane sugar
MY50	<i>S. octosporus</i> Beijerinck	NRRL Y-854	ATCC 2479
MY59	<i>S. octosporus</i> Beijerinck	NCYC 131	
MY49	<i>S. octosporus</i> Beijerinck	CBS 371	Type strain; ATCC 4206, NRRL Y-855; currants

* Abbreviations: ATCC, American Type Culture Collection, Rockville, Md., USA; AWRI, Australian Wine Research Institute, Adelaide, South Australia; CBS, Centraalbureau voor Schimmelcultures (Yeast Division), Delft, The Netherlands; IFO, Institute for Fermentation, Osaka, Japan; JMM, Professor J. M. Mitchison, Department of Zoology, University of Edinburgh, Edinburgh, Scotland; L, Professor U. Leupold, Institute of General Microbiology, University of Bern, Switzerland; NCYC, National Collection of Yeast Cultures, Food Research Institute, Norwich, England; NRRL, Northern Regional Research Center, US Department of Agriculture, Peoria, Illinois, USA; RIVE, Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia; RYCC, Roseworthy Yeast Culture Collection, Roseworthy Agricultural College, Roseworthy, South Australia; UCD, Department of Food Science and Technology, University of California, Davis, California, USA.

Inoculation of test media. Liquid media were inoculated with 0.1 ml of washed cell suspension. Except for plates of MEA required for the study of colonial appearance and sporulation, plates of agar media were inoculated with a matrix of 25 strains using a multipoint inoculator (May & Houghton, 1965).

Colonial and cellular morphology. Colonial appearance and number of spores per ascus were ascertained after 7 d on MEA. Colonial morphology was described using criteria given by Wilson & Miles (1964) and Lodder (1970). Length and width of cells just prior to division (i.e. showing a cell plate) were ascertained after 2 d in PYGM. Formation of hyphae penetrating the agar surrounding an area of growth was ascertained after 7 d on MEA incubated anaerobically (Gas Pak Anaerobic System, BBL).

Growth at different temperatures. Ability to grow at 4 °C (14 d), 10 °C (14 d), 37 °C (7 d), 40 °C (5 d) and 45 °C (5 d) was tested in PYGM.

Growth at pH 3.3. Ability to grow within 10 d was tested on MEA adjusted to pH 3.3.

Growth with various inhibitors. Ability to grow within 10 d was tested on EMM2A agar containing in turn each of the substances listed in Table 3 (nos 28–66 inclusive) at the stated concentrations.

Ability to grow within 10 d was also tested on Bismuth Sulphite Agar (Oxoid) adjusted to pH 5.6.

Assimilation of carbohydrates. Ability to grow within 14 d was tested on EMM2A(G-0) agar containing in turn each of the following filter-sterilized carbohydrates (10 g l^{-1}): dihydroxyacetone, D-fructose, D-galactose, α -D-glucosamine, D-glucose, glycerol, maltose, D-mannose, raffinose, D-ribose, L-sorbose, sucrose, trehalose and xylose.

Fermentation of carbohydrates. Ability to produce gas from carbohydrates within 28 d was tested in EMM2A(G-0) containing in turn each of the following carbohydrates (10 g l^{-1}): D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannose, raffinose and sucrose. Gas formation was detected using a Durham tube in each bottle of medium.

Production of proteases. Digestion of casein was tested after 10 d on EMM2A(G-3) agar containing 30% (w/v) Skimmed Milk Medium (Difco). A clear zone around an area of growth after flooding with 10% (w/v) solution of HgCl_2 was scored as positive.

Liquefaction of gelatin was tested after 10 d in EMM2A containing 12% (w/v) gelatin. Failure of the medium to reform a gel after chilling was scored as positive.

Enzymic activities. Aesculin hydrolysis was ascertained after 10 d on EMM2A(G-3) agar containing 1% (w/v) aesculin and 0.05% (w/v) ferric chloride. Blackening of the medium around an area of growth was scored as positive.

Catalase activity was tested after 10 d on MEA. Effervescence after application of 3% (v/v) hydrogen peroxide was scored as positive.

DNAase activity was tested after 10 d on EMM2A agar containing 0.2% (w/v) DNA (sodium salt, BDH). A clear zone around an area of growth after flooding with 1 M-HCl was scored as positive.

Phosphatase activity was tested after 10 d on EMM2A agar containing 0.01% (w/v) phenolphthalein diphosphate. Pink coloration of growth after exposure to vapour from ammonia solution (sp. gr. 0.88) was scored as positive.

Starch hydrolysis was tested after 10 d on EMM2A(G-3) agar containing 1% (w/v) soluble starch. A clear zone around an area of growth was scored as positive.

Urease activity was ascertained after 5 d on slopes of EMM2A agar containing 2% (w/v) filter-sterilized urea as the sole nitrogen source and 0.12% (w/v) phenol red. A reddening of the medium was scored as positive.

Metabolic end products. Production of acetoin after 10 d in PYGM was tested using method 1 described by Cowan (1974).

Hydrogen sulphide production was ascertained after 10 d in PYGM containing sodium thiosulphate (0.005 g l^{-1}). Blackening of a lead acetate impregnated paper (Cowan, 1974) fixed above the medium was scored as positive.

Reduction of tellurite. Blackening of growth after 10 d on EMM2A agar containing potassium tellurite (0.032 g l^{-1}) was scored as positive.

Numerical methods. Reproducibility was estimated within this study by two methods. Firstly, duplicate cultures of five strains (MY1, MY12, MY23, MY31 and MY48) were maintained and tested independently, the duplicate cultures being indicated in the phenogram (Fig. 1) by the suffix A. Secondly, ten tests (nos 24, 25, 37, 57, 58, 62, 70, 73, 75 and 76) were duplicated for all strains. Test error was estimated using formula 1 of Sneath & Johnson (1972). Vigour values were calculated as the proportion of positive results for each strain.

A data matrix was constructed for the 60 strains and 5 duplicate cultures. Characters that gave either all positive or all negative results were deleted from the computations. The final data matrix contained information from 80 unit characters.

A computer program based upon methods of M. J. Sackin and associates (Leicester University) was used to construct similarity matrices using both the simple matching coefficient (S_{SM}) and the pattern difference (D_P). Phenograms were constructed using average linkage (UPGMA) methods as given by Sneath & Sokal (1973). Cophenetic discrepancies, that is the lack of correlation between respective similarity values in a matrix and its corresponding phenogram, were assessed using the correlation coefficient (r ; see Sneath, 1978). The inter-centroid taxonomic distance between two clusters J and K (d_{JK}) and the mean taxonomic distance of strains of a cluster to their own centroid ($\bar{d}_J$) were calculated using formulae given by Sneath & Sokal (1973) and Bridge & Sneath (1983).

The computer program OVCLUST (Sneath, 1979) was used to calculate the overlap statistics for each pair of clusters in Euclidean space. An index of overlap (V_O), which is based upon the number of strains in each of the two clusters, the inter-centroid distance and the distances of strains to the centroids, was calculated and compared to a critical level of overlap (V_{crit}) by a non-central t -test. A value of 1% was used as the critical level of overlap when comparing each pair of clusters. Since the program OVCLUST allows for a correction to be made for sampling error based on the number of tests, computations were made both with and without this correction.

A Digital Equipment Corporation VAX 11 computer was used for all data processing.

RESULTS AND DISCUSSION

Cluster analysis

Both the S_{SM} and the D_P produced three major clusters, the composition and structure of which were essentially the same with each coefficient. The phenogram derived using the S_{SM} is shown in Fig. 1, the parameters of the three resulting clusters are listed in Table 2 and the characteristics of each cluster are listed in Table 3. A list of the most differential tests for the clusters observed is given in Table 4.

Although differences in vigour may cause more distortion of the clustering obtained with S_{SM} than with D_P (Sneath, 1968; Sneath & Sokal, 1973), the good agreement between the clustering obtained with the two coefficients implies that the observed differences in vigour (Table 2) did not have a significant effect. In addition, the correlation coefficient (r) between a similarity matrix and its corresponding phenogram was 0.9404 for the S_{SM} and 0.880 for the D_P , these values being quite acceptable for a study employing UPGMA. The classification described below is based upon the phenogram derived with the S_{SM} and results using D_P are referred to in the text only where they showed any apparent differences.

Description and identity of clusters

Cluster 1 was named *Schizosaccharomyces pombe*. This cluster contained 41 strains of which 4 were duplicated. These 41 strains consisted of all 32 strains, including the type strain (MY15), of *S. pombe*, 3 strains (MY41, MY42 and MY43) received as *S. mellacei*, *S. mosquensis* and *S. acidodevoratus* respectively, 2 strains (MY8 and MY48) received as the type strain of *S. malidevorans*, and 4 strains (MY9, MY10, MY11 and MY12) received as species of *Schizosaccharomyces*.

Using the S_{SM} , six strains (MY51, MY52, MY53, MY5, MY45 and MY54) were loosely linked at the base of cluster 1, although using D_P only two strains occupied this position. Since all six strains were incorporated at an S_{SM} of at least 83% and because of the large difference in similarity between this cluster and its nearest neighbour (cluster 2), these strains were considered to be part of cluster 1.

The characteristics of cluster 1 (see Table 3) were in general agreement with the findings of Slooff (1970) for *S. pombe*, although there were three notable exceptions. Whereas Slooff (1970) reported that members of this species fermented raffinose and failed to or only weakly assimilated glycerol, and that certain strains liquefied gelatin, we found that 91% of strains failed to ferment raffinose, 93% of strains assimilated glycerol, and three strains (MY16, MY32 and MY53) previously designated *S. liquefaciens*, failed to liquefy gelatin. The concentration (approximately 17 mM) of raffinose used in this study may have been insufficient to allow visible gas formation given the size of the inoculum and the incubation period, particularly since other workers have used concentrations of 50 mM (Barnett *et al.*, 1979), 68 mM (Lodder, 1970) and 200 mM (Beech *et al.*, 1968). Unfortunately it was not possible to retest the strains described here at higher concentrations of carbohydrate. The higher proportion of strains assimilating glycerol was not surprising since the enzymes involved in glycerol utilization by *S. pombe* were described recently (May & Sloan, 1981; May *et al.*, 1982) and were present in all members of cluster 1 (G. Vasiliadis, personal communication). The failure of strains formerly designated *S. liquefaciens* to liquefy gelatin may reflect either loss of the property during maintenance or lack of expression due to the composition of the medium and/or the cultural conditions employed.

The two lines of the type strain of *S. malidevorans* were well separated in cluster 1 (S_{SM} = 87%, D_P = 0.13). Line MY8, obtained from the Australian Wine Research Institute, showed properties similar to those described by Rankine & Fornachon (1964): it grew well at 37 °C, assimilated maltose and was homothallic. Line MY48, obtained from the Centraalbureau voor Schimmelcultures, showed properties closer to those described by Sipiczki *et al.* (1982): it grew poorly at 37 °C, failed to assimilate maltose and was heterothallic. Like the line used by Sipiczki *et al.* (1982), line MY48 probably originated from a stable mating-type mutant (see Leupold, 1970) and this may have been responsible for the physiological differences between MY8 and MY48.

Fig. 1. UPGMA phenogram based on S_{SM} .

Table 2. *Parameters of clusters*

The mean vigour for a cluster is the average of all of the individual strain vigour values in the cluster, with SD indicating the standard deviation of these values; $\bar{d}_j$ is the mean taxonomic distance of strains of a cluster to their own centroid; and d_{JK} is the inter-centroid taxonomic distance between clusters *J* and *K*.

	Cluster 1	Cluster 2	Cluster 3
No. of strains	45	8	12
Mean vigour	0.483	0.371	0.390
SD	0.028	0.052	0.039
$\bar{d}_j$	0.252	0.306	0.324
d_{JK} { cluster 1	0.0	.	.
cluster 2	0.361	0.0	.
cluster 3	0.417	0.361	0.0

The inclusion of *S. malidevorans* within cluster 1 was expected since this species and *S. pombe* were similar in the chemotaxonomic studies of Yamada *et al.* (1973), Stewart & Widanapratirana (1974) and Claisse & Simon-Becam (1978). Recently, Sipiczki *et al.* (1982) proposed *S. malidevorans* as a variety of *S. pombe* on the basis of results from interfertility studies. Our results suggest that there is insufficient distinction between the two to justify variety status for *S. malidevorans* and that it should be a member of the species *S. pombe*. We found no basis for separation of subgroups or biotypes within the clusters (see below: *Overlap*); this is consistent with the decision of Slooff (1970) not to raise to either species or variety level those strains which fermented L-malic acid.

Cluster 2 was named *Schizosaccharomyces japonicus*. This cluster contained eight strains and consisted of all four strains, including the type strain (MY13), of *S. japonicus* var. *japonicus*, and all four strains, including the type strain (MY14), of *S. japonicus* var. *versatilis*. The constituent strains formed a loose but distinct cluster ($S_{SM} = 78\%$, $D_P = 0.20$).

The characteristics of the cluster were in broad agreement with those given by Slooff (1970) for *S. japonicus*, although fewer positive results were obtained for assimilation and fermentation of carbohydrates. As suggested previously, this may have been due to the lower concentration of carbohydrates used in this study. The recommended separation of this species into two varieties (Slooff, 1970) was not justified by either S_{SM} or D_P . Indeed, the inter-variety pairings in the cluster had a mean S_{SM} equal to 82% which was higher than that for either of the two groups of intra-variety pairings in the cluster (mean $S_{SM} = 81\%$ for both). Furthermore, the type strains of *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* (MY13 and MY14 respectively) formed a pair at an S_{SM} equal to 86%; and the equal highest similarity in the cluster ($S_{SM} = 90\%$) was between MY36 and MY61, strains of *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* respectively. Consequently, on the basis of overall similarity, there is no justification for the division of the cluster into sub-clusters corresponding to the varieties *japonicus* and *versatilis*.

Cluster 3 was named *Schizosaccharomyces octosporus*. This cluster contained 11 strains (one of which was duplicated) and consisted of all 10 strains, including the type strain (MY49), of *S. octosporus* and the type strain (MY26) of *S. slooffiae*. The constituent strains were clustered loosely ($S_{SM} = 75\%$, $D_P = 0.24$), this lack of cohesion being exemplified by MY23 and its duplicate culture MY23A which only joined at an S_{SM} of 79%. Nevertheless, the cluster was sufficiently well separated from its nearest neighbour (cluster 2) to be regarded as distinct.

The characteristics of the cluster were in broad agreement with those given by Slooff (1970) for *S. octosporus*. A notable exception was fermentation of maltose which was reported by Slooff (1970) to be positive, whereas in this study as many as 83% of the strains in the cluster failed to ferment maltose. Again, this result may have been due to the concentration of carbohydrate used. This inconsistency is inconvenient since inability to ferment maltose was the principal feature distinguishing *S. slooffiae* from *S. octosporus* (Kumbhojkar, 1972); nevertheless, the presence of the type strain of *S. slooffiae* (MY26) near the centre of the cluster indicates that its species status is not justified and that it should be included in *S. octosporus*.

Table 3. *Characteristics of the clusters*

Character	Percentage of strains positive		
	Cluster 1	Cluster 2	Cluster 3
1. White colonies	100	63	8
2. Buff colonies	0	37	92
3. Convex colonies	73	63	8
4. Umbonate colonies	0	37	42
5. Conical colonies	27	0	50
6. Entire colonies	100	100	33
7. Crenated colonies	0	0	67
8. Rough colonies	0	13	92
9. Hyphae penetrating agar	0	100	0
10. Colony diameter <2 mm	49	88	42
11. Colony diameter 2-3 mm	49	12	58
12. Colony diameter >3 mm	2	0	0
13. Cell length <8 μ m	15	0	17
14. Cell length 8-10 μ m	56	25	58
15. Cell length >10 μ m	29	75	25
16. Cell width <3 μ m	20	0	0
17. Cell width 3-4.5 μ m	80	0	0
18. Cell width >4.5 μ m	0	100	100
19. Asci containing <5 spores	100	37	8
20. Asci containing 5-7 spores	0	63	33
21. Asci containing >7 spores	0	0	59
22. Growth at 4 °C	4	0	0
23. Growth at 10 °C	84	75	8
24. Growth at 37 °C	96	100	75
25. Growth at 40 °C	60	88	25
26. Growth at 45 °C	0	0	0
27. Growth at pH 3.3	100	88	58
<i>Growth with:</i>			
28. Brilliant green (0.01 g l ⁻¹)	0	0	0
29. Caesium chloride (0.005 g l ⁻¹)	100	75	83
30. Cobalt nitrate (0.005 g l ⁻¹)	96	50	25
31. Cobalt nitrate (0.05 g l ⁻¹)	2	0	0
32. Copper sulphate (0.005 g l ⁻¹)	56	13	25
33. Crystal violet (0.004 g l ⁻¹)	98	75	83
34. Crystal violet (0.1 g l ⁻¹)	20	0	8
35. Glucose (500 g l ⁻¹)	100	0	100
36. Glucose (600 g l ⁻¹)	98	0	100
37. Horse blood (50 ml l ⁻¹)	84	88	67
38. Lead acetate (0.005 g l ⁻¹)	100	100	92
39. Lead acetate (0.05 g l ⁻¹)	98	50	67
40. Lithium chloride (0.005 g l ⁻¹)	98	75	83
41. Lithium chloride (0.01 g l ⁻¹)	98	62	67
42. Lysozyme (0.05 g l ⁻¹)	98	88	75
43. Lysozyme (0.1 g l ⁻¹)	98	75	67
44. Mercuric chloride (0.005 g l ⁻¹)	0	0	0
45. Ox bile (10 g l ⁻¹)	100	100	100
46. Ox bile (100 g l ⁻¹)	27	25	67
47. Potassium tellurite (0.032 g l ⁻¹)	100	100	92
48. Potassium tellurite (0.32 g l ⁻¹)	0	0	0
49. Rose bengal (0.05 g l ⁻¹)	100	13	58
50. Rose bengal (0.5 g l ⁻¹)	49	0	0

Test performance and strain variability

Whereas only 19% of the tests listed by Barnett *et al.* (1979) were differential for fission yeasts, 76% of our set of tests were significantly differential (i.e. between 10% and 90% of all strains were positive for a given test). This improvement was almost wholly due to the inclusion of tests for resistance, characteristics seldom used in yeast classification (Lodder, 1979; Barnett *et al.*, 1979), but which are widely used in numerical classification of bacteria (e.g. Goodfellow *et al.*,

Table 3 (*continued*)

Character	Percentage of strains positive		
	Cluster 1	Cluster 2	Cluster 3
51. Silver nitrate (0.005 g l ⁻¹)	7	0	0
52. Sodium arsenate (0.02 g l ⁻¹)	82	50	50
53. Sodium arsenate (0.05 g l ⁻¹)	36	13	25
54. Sodium azide (0.1 g l ⁻¹)	42	25	0
55. Sodium azide (1 g l ⁻¹)	0	0	0
56. Sodium benzoate (1 g l ⁻¹)	89	13	0
57. Sodium benzoate (10 g l ⁻¹)	9	0	0
58. Sodium chloride (30 g l ⁻¹)	24	13	92
59. Sodium chloride (40 g l ⁻¹)	2	0	92
60. Sodium chloride (60 g l ⁻¹)	0	0	75
61. Sodium chloride (100 g l ⁻¹)	0	0	42
62. Sodium chloride (150 g l ⁻¹)	0	0	17
63. Sodium dodecyl sulphate (1 g l ⁻¹)	0	0	0
64. Sodium hydrogen selenite (0.032 g l ⁻¹)	58	0	0
65. Tetrazolium salt (0.032 g l ⁻¹)	82	75	83
66. Zinc acetate (0.005 g l ⁻¹)	98	75	75
67. Growth on Bismuth Sulphite Agar (pH 5.6)	18	0	0
<i>Assimilation of carbohydrates (10 g l⁻¹):</i>			
68. Dihydroxyacetone	80	0	0
69. D-Fructose	100	88	83
70. D-Galactose	0	0	0
71. α -D-Glucosamine	0	0	0
72. D-Glucose	100	100	100
73. Glycerol	93	0	0
74. Maltose	96	100	100
75. D-Mannose	89	50	67
76. Raffinose	96	25	8
77. D-Ribose	0	0	0
78. L-Sorbose	0	0	0
79. Sucrose	100	100	83
80. Trehalose	0	0	0
81. Xylose	0	0	0
<i>Fermentation of carbohydrates (10 g l⁻¹):</i>			
82. D-Fructose	96	75	75
83. D-Galactose	0	0	0
84. D-Glucose	100	100	100
85. Glycerol	0	0	0
86. Maltose	93	100	17
87. D-Mannose	82	63	50
88. Raffinose	9	50	0
89. Sucrose	100	38	17
90. Casein digestion	0	13	0
91. Gelatin liquefaction	0	0	0
92. Aesculin hydrolysis	0	50	0
93. Catalase	100	100	100
94. DNAase	0	0	0
95. Phosphatase (pH 5.6)	96	100	58
96. Starch hydrolysis	0	0	0
97. Urease	96	75	92
98. Acetoin production	4	0	58
99. H ₂ S production	47	50	92
100. Reduction of potassium tellurite	100	63	75

1976; Piot *et al.*, 1980; Schofield & Schaal, 1981; Bridge & Sneath, 1983; Williams, *et al.*, 1983).

Results from the duplicated tests showed differences between 1.6% and 13.1%, the mean difference being 8.5% (SE = ± 0.92). This is within the range regarded as acceptable in a study of this kind (Sneath & Johnson, 1972).

In bacterial taxonomy characters derived from morphological features have often been shown

Table 4. *Plus-minus table of the most differential characters for all three clusters*

Character	Cluster 1	Cluster 2	Cluster 3
1. White colonies	+	v	—
2. Buff colonies	—	v	+
9. Hyphae penetrating agar	—	+	—
17. Cell width 3–4.5 μm	+	—	—
18. Cell width >4.5 μm	—	+	+
19. Asci containing <5 spores	+	v	—
23. Growth at 10 °C	+	v	—
35. Growth with glucose (500 g l ⁻¹)	+	—	+
36. Growth with glucose (600 g l ⁻¹)	+	—	+
49. Growth with rose bengal (0.05 g l ⁻¹)	+	—	v
56. Growth with sodium benzoate (1 g l ⁻¹)	+	—	—
58. Growth with sodium chloride (30 g l ⁻¹)	v	—	+
59. Growth with sodium chloride (40 g l ⁻¹)	—	—	+
68. Assimilation of dihydroxyacetone	+	—	—
73. Assimilation of glycerol	+	—	—
76. Assimilation of raffinose	+	v	—
86. Fermentation of maltose	+	+	—
89. Fermentation of sucrose	+	v	—

—, $\leq 20\%$ of strains positive; v, 21–79% of strains positive; +, $\geq 80\%$ of strains positive.

to be poorly reproducible (Sneath & Collins, 1974). Yeast taxonomy is governed by the articles of the International Code of Botanical Nomenclature (Stafleu, 1978) and so morphological features must be considered. In this study, the use of standard morphological descriptions and the distinctive features of the organisms enabled reproducible results to be obtained.

Results from the duplicated cultures included in the numerical analysis showed a mean S_{SM} of 89.1% (SE = ± 2.6) when compared with their parent cultures. Four of the five pairs showed S_{SM} values equal to or greater than 87%, whereas for the fifth pair (MY23 and MY23A) the value was only 79%. Nevertheless, the mean corresponds to a test error of 5.9%, which is considered acceptable (Sneath & Johnson, 1972).

We have also observed S_{SM} values as low as 87% between different lines of the same strain in two cases in addition to that of *S. malidevorans*. The first case involves MY5, MY6 and MY56 which are different lines of *S. pombe* NCYC 132. This strain appears to be inherently unstable: Mitchison & Creanor (1971) reported differences in morphology and timing of DNA synthesis for two separate lines and Mahadi (1977) observed that, in contrast to MY5, MY6 was unable to assimilate glycerol and produced less acid phosphatase following derepression. The second case involves MY16 and MY32 which are different lines of a strain of *S. liquefaciens* (CBS 1042 and IFO 0358 respectively) maintained separately since 1946. These two lines showed consistent differences in six tests, including cell size, ability to assimilate mannose and ability to grow in the presence of sodium chloride (30 g l⁻¹). Similar persistent variations in yeast cultures were observed by Stahl & Esser (1979) who concluded that accumulation of mutants during prolonged subculturing was responsible. Such variations may have significant implications for existing yeast taxonomies, particularly those in which disproportionate weight is given to a particular character (Beech *et al.*, 1968; Griffiths, 1981; Payne *et al.*, 1982).

Overlap

For each pair of clusters, a *t*-test showed that overlap was significantly less than 1% at a confidence level of 99%. Varying the number of test results used for correction of sampling error, as described by Bridge & Sneath (1983), showed no significant alteration in the confidence level. The same procedure was used to show that no apparent subclusters were sufficiently distinct to justify subdivision of any cluster.

General taxonomic considerations and conclusions

Reproduction by binary fission sets members of the genus *Schizosaccharomyces* apart from all other ascosporogenous yeasts (Lodder, 1970). However, this character was not considered

directly in this study nor were representatives of other genera of yeasts included. Given these restraints, it was not possible to consider the position of *Schizosaccharomyces* within the overall context of yeast classification.

The proposal of Kudriavzev (see Slooff, 1970) to divide fission yeasts into two genera, *Schizosaccharomyces* (consisting of the four-spored *S. pombe* and *S. malidevorans*) and *Octosporomyces* (consisting of the eight-spored *S. japonicus* and *S. octosporus*), was supported by the finding of coenzyme Q₁₀ in *S. pombe* and *S. malidevorans* and of coenzyme Q₉ in *S. japonicus* and *S. octosporus* (Yamada *et al.*, 1973). Although our results revealed the distinctness of *S. pombe* and *S. malidevorans* (cluster 1) from *S. japonicus* (cluster 2) and *S. octosporus* (cluster 3), in view of the reported low similarity values between yeast taxa generally accepted as species within the same genus (Campbell, 1973, 1974), further studies are required to establish the precise taxonomic status of the clusters.

The results of this study show that the genus *Schizosaccharomyces* Lindner consists of three distinct taxa corresponding to the species *S. pombe* Lindner, *S. japonicus* Yukawa & Maki and *S. octosporus* Beijerinck as described by Slooff (1970). Although each species showed some loosely linked members, there was no evidence for the subdivision of any species into varieties.

We wish to thank Dr E. Minarik (Research Institute for Viticulture and Enology, Bratislava), Professor J. M. Mitchison (Department of Zoology, University of Edinburgh), Dr P. Monk (Australian Wine Research Institute, South Australia) and Dr A. S. J. Yap (Roseworthy Agricultural College, South Australia) for the gift of strains held in their collections.

REFERENCES

- BARNETT, J. A., PAYNE, R. W. & YARROW, D. (1979). *A Guide to Identifying and Classifying Yeasts*. Cambridge University Press.
- BEECH, F. W., DAVENPORT, R. R., GOSWELL, R. W. & BURNETT, J. K. (1968). Two simplified schemes for identifying yeast cultures. In *Identification Methods for Microbiologists*, vol. 2B, pp. 151–175. Edited by B. M. Gibbs & D. A. Shapton. London: Academic Press.
- BRIDGE, P. D. & SNEATH, P. H. A. (1983). Numerical taxonomy of *Streptococcus*. *Journal of General Microbiology* **129**, 565–597.
- CAMPBELL, I. (1972). Numerical analysis of the genera *Saccharomyces* and *Kluyveromyces*. *Journal of General Microbiology* **73**, 279–301.
- CAMPBELL, I. (1973). Numerical analysis of *Hansenula*, *Pichia* and related yeast genera. *Journal of General Microbiology* **77**, 427–441.
- CAMPBELL, I. (1974). Methods of numerical taxonomy for various genera of yeasts. *Advances in Applied Microbiology* **17**, 135–156.
- CAMPBELL, I. (1975). Numerical analysis and computerized identification of the yeast genera *Candida* and *Torulopsis*. *Journal of General Microbiology* **90**, 125–132.
- CLAISSE, M. L. & SIMON-BECAM, A.-M. (1978). Cytochrome *c* from *Schizosaccharomyces pombe*. I. Purification, spectral properties, and amino-acid composition. *European Journal of Biochemistry* **86**, 399–406.
- CONTI, S. F. & NAYLOR, H. B. (1959). Electron microscopy of ultrathin sections of *Schizosaccharomyces octosporus*. I. Cell division. *Journal of Bacteriology* **78**, 868–877.
- COWAN, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.
- GOODFELLOW, M., AUSTIN, B. & DICKINSON, C. H. (1976). Numerical taxonomy of some yellow-pigmented bacteria isolated from plants. *Journal of General Microbiology* **97**, 219–233.
- GRIFFITHS, A. J. (1981). A nomenclature of the yeasts. *Antonie van Leeuwenhoek* **47**, 547–563.
- KOCKOVA-KRATOCHVÍLOVA, A., SLAVIKOVA, E. & JENSEN, V. (1978). Numerical taxonomy of the yeast genus *Debaryomyces* Lodder & Kreger-van Rij. *Journal of General Microbiology* **104**, 257–268.
- KUMBHOJKAR, M. S. (1972). *Schizosaccharomyces slooffiae* Kumbhojkar, a new species of osmophilic yeasts from India. *Current Science* **41**, 151–152.
- LEUPOLD, U. (1970). Genetical methods for *Schizosaccharomyces pombe*. *Methods in Cell Physiology* **4**, 169–177.
- LODDER, J. (editor) (1970). *The Yeasts – a Taxonomic Study*, 2nd edn. Amsterdam: North-Holland Publishing Co.
- LODDER, J. & KREGER-VAN RIJ, N. J. W. (editors) (1952). *The Yeasts – a Taxonomic Study*, 1st edn. Amsterdam: North-Holland Publishing Co.
- MAHADI, N. M. B. (1977). *Control of production of sucrase and maltase in Schizosaccharomyces pombe*. PhD thesis, Monash University, Australia.
- MAY, J. W. & HOUGHTON, R. H. (1965). A simple multipoint inoculator. *Laboratory Practice* **14**, 168.
- MAY, J. W. & SLOAN, J. (1981). Glycerol utilization by *Schizosaccharomyces pombe*: dehydrogenation as the initial step. *Journal of General Microbiology* **123**, 183–185.
- MAY, J. W., MARSHALL, J. H. & SLOAN, J. (1982). Glycerol utilization by *Schizosaccharomyces pombe*: phosphorylation of dihydroxyacetone by a specific kinase as the second step. *Journal of General Microbiology* **128**, 1763–1766.

- MITCHISON, J. M. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. *Methods in Cell Physiology* **4**, 131–165.
- MITCHISON, J. M. & CREANOR, J. (1971). Further measurements of DNA synthesis and enzyme potential during cell cycle of fission yeast *Schizosaccharomyces pombe*. *Experimental Cell Research* **69**, 244–247.
- PAYNE, R. W., YARROW, D. & BARNETT, J. A. (1982). The construction by computer of a diagnostic key to the genera of yeasts and other such groups of taxa. *Journal of General Microbiology* **128**, 1265–1277.
- PIOT, P., VAN DYCK, E., GOODFELLOW, M. & FALKOW, S. (1980). A taxonomic study of *Gardnerella vaginalis* (*Haemophilus vaginalis*) Gardner and Dukes 1955. *Journal of General Microbiology* **119**, 373–396.
- RANKINE, B. C. & FORNACHON, J. C. M. (1964). *Schizosaccharomyces malidevorans* sp. n., a yeast decomposing L-malic acid. *Antonie van Leeuwenhoek* **30**, 73–75.
- SCHOFIELD, G. M. & SCHAAL, K. P. (1981). A numerical taxonomic study of members of the *Actinomycetaceae* and related taxa. *Journal of General Microbiology* **127**, 237–259.
- SIPICZKI, M., KUCSERA, J., ULASZEWSKI, S. & ZSOLT, J. (1982). Hybridization studies by crossing and protoplast fusion within the genus *Schizosaccharomyces* Lindner. *Journal of General Microbiology* **128**, 1989–2000.
- SLOOFF, W. C. (1970). *Schizosaccharomyces* Lindner. In *The Yeasts – a Taxonomic Study*, pp. 733–755. Edited by J. Lodder. Amsterdam: North-Holland Publishing Co.
- SNEATH, P. H. A. (1968). Vigour and pattern in taxonomy. *Journal of General Microbiology* **54**, 1–11.
- SNEATH, P. H. A. (1978). Classification of microorganisms. In *Essays in Microbiology* pp. 9/1–9/31. Edited by J. R. Norris & M. H. Richmond. Chichester: John Wiley.
- SNEATH, P. H. A. (1979). BASIC program for a significance test for two clusters in Euclidean space as measured by their overlap. *Computers and Geosciences* **5**, 143–155.
- SNEATH, P. H. A. & COLLINS, V. G. (1974). A study in test reproducibility between laboratories: report of a *Pseudomonas* working party. *Antonie van Leeuwenhoek* **40**, 481–527.
- SNEATH, P. H. A. & JOHNSON, R. (1972). The influence on numerical taxonomic similarities of errors in microbiological tests. *Journal of General Microbiology* **72**, 377–392.
- SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy*. San Francisco: W. H. Freeman.
- STAFLEU, F. A. (editor) (1978). *International Code of Botanical Nomenclature*. Utrecht: Bonn, Scheltema & Holkema.
- STAHL, U. & ESSER, K. (1979). Inconsistency in the species concept for yeasts due to mutations during vegetative growth. *European Journal of Applied Microbiology and Biotechnology* **8**, 271–278.
- STEWART, D. J. & WIDANAPATIRANA, S. (1974). 'Finger-print' analysis of cell wall proteins from different yeast species after trypsinization. *Microbios* **9**, 167–172.
- WILLIAMS, S. T., GOODFELLOW, M., ALDERSON, G., WELLINGTON, E. M. H., SNEATH, P. H. A. & SACKIN, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *Journal of General Microbiology* **129**, 1743–1813.
- WILSON, G. S. & MILES, A. A. (1964). *Topley & Wilson's Principles of Bacteriology and Immunity*, vol 1, 5th edn. London: Arnold.
- YAMADA, Y., ARIMOTO, M. & KONDO, K. (1973). Coenzyme Q system in the classification of the ascosporogenous yeast genus *Schizosaccharomyces* and yeast-like genus *Endomyces*. *Journal of General and Applied Microbiology* **19**, 353–358.