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The application of whole-cell protein electrophoresis for the classification and identification of basidiomycetous yeast species

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Abstract

The relationships among 65 basidiomycetous yeast strains were determined by one-dimensional electrophoresis of SDS-solubilized whole-cell proteins. Protein profiles were compared by the Pearson product moment correlation coefficient (r). The strains investigated represented species from the genera *Cystofilobasidium, Filobasidium, Filobasidiella, Kondoa, Leucosporidium, Mrakia* and *Rhodosporidium.* Except for the genus *Mrakia*, all species constituted separate protein electrophoretic clusters. The species of the genus *Mrakia* (*M. frigida, M. gelida, M. nivalis* and *M. stokesii*) show highly similar protein patterns, suggesting that these four species may be synonymous. Strains of two varieties of *Filobasidiella neoformans, F. neoformans* var. *neoformans* and *F. neoformans* var. *bacillispora*, could not be differentiated by protein electrophoresis.

For the delineation of the protein electrophoretic clusters of the yeasts studied, literature data relying on other criteria, such as DNA base composition, carbon source utilization patterns, enzymatic protein electrophoregrams, ubiquinone systems, DNA-DNA homology and rRNA sequence data were used. It was demonstrated that a database of SDS-protein patterns provides a valuable tool for the identification of yeasts.

Introduction

Since the appearance of the first Delft monographs, the number of characters for the standard description of yeast species has increased considerably. Traditional taxonomic methods based upon morphological and physiological approaches have many shortcomings as pointed out by Phaff & Price (1977). Chemotaxonomic techniques, such as DNA base composition (Storck et al. 1969; Nakase & Komagata 1971; Hamamoto et al. 1986), DNA-DNA homology (Aulakh et al. 1981; Hamamoto et al. 1987), ubiquinone systems (Yamada & Kondo 1973, 1976), zymogram comparison (Yamazaki & Komagata 1981; Yamada & Matsumoto 1988) and cellular carbohydrate compositions (Sugiyama et al. 1985) are promising tools for the classification and identification of yeasts.

Yet, strains of some asporogenous species differ sometimes considerably in their % GC and might therefore belong to different species (Hamamoto et al. 1986). DNA-DNA homology and the production of a zygote (as an indicator of conspecificity) do not always give unequivocal delineation of species (Hamamoto et al. 1987; van der Walt & Johanssen 1979). Consequently, there is a need for additional and simplified approaches for species delimitation and identification.

Table 1. List of strains studied and their sources of isolation.

Species name and MUCL strain number[a]	Other strain designation[b]	Source[b]
Cystofilobasidium bisporidii (Fell et al.) Oberwinkler et Bandoni		
30309	CBS 6346	J. W. Fell; Antarctic Ocean; holotype, mating type A1B1
30310	CBS 6347	J. W. Fell; Antarctic Ocean; allotype, mating type A2B2
30311	CBS 6348	J. W. Fell; monosporidial isolate from mixture of 35-32 (CBS 6346) x 23-1 (CBS 6347); mating type A1B2
Cystofilobasidium capitatum (Fell et al.) Oberwinkler et Bandoni		
30321	CBS 6358	J. W. Fell; zooplancton, Southern Indian Ocean; holotype, self fertile
30354	CBS 6230	A. Bruce; sea; self fertile
30361	CBS 6723	R. W. M. Buhagiar; rhubarb, UK; self fertile
Cystofilobasidium infirmominiatum (Fell et al.) Hamamoto et al.		
30407	CBS 323	K. Okunuki; atmosphere, Japan; holotype, mating type A1
30426	CBS 2205	deep-frozen vegetables, The Netherlands; mating type A2
30450	CBS 6352	J. W. Fell; Antarctic Ocean; allotype, mating type A3
Filobasidiella neoformans Kwon-Chung var. *bacillispora* Kwon-Chung		
30449	CBS 6289	R. Vanbreuseghem; human cerebral cryptococcosis, Zaïre; mating type a
30454	CBS 6955	K. J. Kwon-Chung; spinal fluid, USA; holotype, mating type a
Filobasidiella neoformans Kwon-Chung var. *neoformans*		
27747	DSM 70219	no information
30453	CBS 6886	ATCC; pigeon droppings, Denmark; holotype, mating type a
Filobasidiella neoformans Kwon-Chung		
30412	CBS 888	Leonian; mating type alpha
Filobasidium capsuligenum (Fell et al.) Rodrigues de Miranda		
30295	CBS 6122	G. Kraepelin; forest soil, Germany; diploid
30296	CBS 4736	J. P. van der Walt; wine cellar, South Africa; holotype, mating type alpha
30297	CBS 1906	Nagao Institute; sake-moto, Japan; holotype, mating type a
30658	CBS 4381	F. W. Beech; cider, UK; mating type a
Filobasidium floriforme Olive		
30246	CBS 6240	L. S. Olive; dead florets of *Erianthus giganteus*, South Carolina; diploid
30247	CBS 6241	L. S. Olive; *Hibiscus siriaca*, Georgia; holotype, mating type alpha
30248	CBS 6242	L. S. Olive; *Hibiscus siriaca*, Georgia; holotype, mating type a
Filobasidium uniguttulatum Kwon-Chung		
27743	DSM 70225	skin, man
30417	CBS 1730	F. Zach; infected finger nail, Austria; holotype, mating type a
30430	CBS 2770	A. Beute; white patches in mouth, The Netherlands; mating type alpha
Kondoa malvinella (Fell et Hunter) Yamada et al.		
30317	CBS 6082	J. W. Fell; Antarctic Ocean; holotype, mating type A1
30353	CBS 6083	J. W. Fell; Antarctic Ocean; allotype, mating type A2
Leucosporidium antarcticum Fell et al.		
30352	CBS 5942	J. W. Fell; Weddell Sea, Joinville Island; holotype, self fertile
30360	CBS 6581	J. W. Fell; Antarctic Ocean; mating type A1
30363	CBS 7009	ATCC; seawater; allotype, mating type A2
Leucosporidium scottii Fell et al.		
28629	CBS 5930	J. W. Fell; Antarctic Ocean; holotype, mating type A2B2
29885	CBS 614	W. J. Scott; soil, Australia; self fertile
30299	CBS 8162	C. Ramirez; rotten trunk of *Eucryphia cordifolia*, Chile; mating type A1B2
30300	CBS 2281	W. J. Scott; chilled beef, Australia; mating type A2B2
30332	CBS 2300	E. Eilertsen; atmosphere, Norway; mating type A1B2
30350	CBS 5932	J. W. Fell; Antarctic Ocean; self fertile
Mrakia frigida (Fell et al.) Yamada et Komagata		
30339	CBS 5270	M. E. di Menna; Antarctic soil; holotype
30340	CBS 5271	M. E. di Menna; Antarctic soil
Mrakia gelida (Fell et al.) Yamada et Komagata		
30341	CBS 5273	M. E. di Menna; Antarctic soil
30342	CBS 5275	M. E. di Menna; Antarctic soil
30662	CBS 5272	M. E. di Menna; Antarctic soil; holotype
Mrakia nivalis (Fell et al.) Yamada et Komagata		
30337	CBS 5266	M. E. di Menna; Antarctic soil; holotype
30338	CBS 5268	M. E. di Menna; Antarctic soil
30348	CBS 5915	H. J. Phaff; snow, Antarctica

Table 1. Continued.

Species name and MUCL strain number[a]	Other strain designation[b]	Source[b]
Mrakia stokesii (Fell et al.) Yamada et Komagata		
30346	CBS 5707	J. P. van der Walt; Bouvet Island
30681	CBS 5917	H. J. Phaff; snow, Antarctica; holotype
Rhodosporidium dacryoidum Fell et al.		
30319	CBS 6353	J. W. Fell; Antarctic Ocean; holotype, mating type A1B1
30357	CBS 6356	J. W. Fell; Antarctic Ocean; allotype, mating type A1B2
30364	CBS 7142	J. W. Fell; sea; mating type A2B2
Rhodosporidium diobovatum Newell et Hunter		
30314	CBS 6085	J. W. Fell; sea, USA; holotype, mating type A1
30329	CBS 994	O. Verona; soil, Italy; mating type A1
30335	CBS 4213	R. A. Kilpatrick; clover *Trifolium repens*, USA; mating type A2
30612	CBS 6084	J. W. Fell; sea, USA; allotype, mating type A2
30682	CBS 5540	J. Villoutreix; cherry blossom, France; mating type A2
Rhodosporidium paludigenum Fell et Statzell Tallman		
30312	CBS 6567	J. W. Fell; mangrove *Rhizophora* mangle swamp, USA; allotype, mating type A2
30313	CBS 6565	J. W. Fell; mangrove *Rhizophora* mangle swamp, USA; mating type A1
30336	CBS 4477	D. G. Ahearn; water, USA; mating type A2
Rhodosporidium sphaerocarpum Newell et Fell		
30298	CBS 5939	J. W. Fell; water, Antarctica; holotype, mating type A2
30306	CBS 5940	J. W. Fell; water, Antarctica; allotype, mating type A1
30351	CBS 5941	J. W. Fell; water, Antarctica; paratype, self fertile
Rhodosporidium toruloides Banno		
11965		no information
28631	CBS 6016	I. Banno; conjugated strain between IFO 0559 and IFO 0880
30249	CBS 14	E. Rennerfelt; conifer pulp, Sweden; holotype, mating type A1
30250	CBS 349	K. Okunuki; soil, Japan; allotype, mating type A2
30328	CBS 350	E. Rennerfelt; conifer pulp, Sweden; mating type A1
30347	CBS 5745	S. Goto; soil, Japan; mating type A2

[a]MUCL, Mycothèque de l'Université Catholique de Louvain, Laboratoire de Mycologie Systématique et Appliquée, UCL Louvain-la-Neuve, Belgium.

[b]Abbreviations: ATCC, American Type Culture Collection, Rockville, Maryland, USA; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of Germany; IFO, Institute for Fermentation, Osaka, Japan.

In a preceding paper, protein electrophoretic fingerprinting has been successfully used for classification and identification of species from the genus *Candida* (Vancanneyt et al. 1991). In the present paper, the results of comparative protein electrophoresis in the genera *Cystofilobasidium, Filobasidium, Filobasidiella, Kondoa, Leucosporidium, Mrakia* and *Rhodosporidium* are presented in an attempt to use this technique as a chemotaxonomic criterion for the systematics of basidiomycetous yeasts. Numerical analysis of protein patterns was used to assist the grouping of large numbers of strains. Literature data were used to evaluate the results.

Materials and methods

Strains studied

Nine strains of three *Cystofilobasidium* species, ten strains of three *Filobasidium* species, five strains of one *Filobasidiella* species, two strains of one *Kondoa* species, nine strains of two *Leucosporidium* species, ten strains of four *Mrakia* species and twenty strains of five *Rhodosporidium* species were used in this study (Table 1). The type strain for all the species studied was included. All strains were obtained from the Mycothèque de l'Université Catholique de Louvain-la-Neuve (MUCL), Belgium.

Cultivation of the cells

Two days old cultures, grown on MYA2 agar slants (per liter distilled water: 20 g malt extract, 3 g yeast extract, 25 g agar, pH 5.5–5.7) at 23° C (*Kondoa* and *Mrakia* strains at 12° C), were suspended in 5 ml sterile 0.025 M phosphate buffer (pH 5.6). One ml of this cell suspension was inoculated in a 250 ml Erlenmeyer containing 40 ml GYPP medium (per liter distilled water: 20 g glucose, 5 g yeast extract, 10 g peptone, 0.025 M phosphate buffer, pH 5.6). Cultures were incubated in a shaking water bath at 23° C (*Kondoa* and *Mrakia* strains at 12° C). Cells were harvested at the logarithmic growth phase and washed three times with NaPBS buffer (per liter bidistilled water: 40.5 ml 0.2 M $Na_2HPO_4.12H_2O$, 9.5 ml 0.2 M $NaH_2PO_4.H_2O$, 8 g NaCl).

Preparation of cell-free extracts

Washed cells (100 mg wet weight) were suspended in 0.9 ml sample treatment buffer (STB; 0.75 g Tris hydroxymethyl methylamine, 5 ml mercaptoethanol, 10 ml glycerol, double distilled water up to 1000 ml) and the ice-cooled suspension was sonicated for 3 min at 50 W with a Labsonic 2000 apparatus (B. Braun, Melsungen AG, Germany) using a needle probe tip. Sonicated samples were treated with sodium dodecyl sulphate (0.1 ml, 20% SDS), mixed, heated for 10 min at 95° C, cooled in ice and centrifuged. The resultant supernatant was stored at − 80° C.

Polyacrylamide gel electrophoresis (PAGE) of proteins

SDS-PAGE of the whole-cell proteins was performed as described by Kiredjian et al. (1986).

Computation

Stained protein electrophoretic patterns were scanned using a LKB 2202 laser densitometer linked to an Apple IIe microcomputer. Densitometric records of 1000 points were normalized and reduced to 400 points as described by Pot et al. (1989). Numerical analysis was done on a Siemens BS2000 mainframe. Calculation of similarity between traces (using points 10 to 320) was performed by the Pearson product moment correlation coefficient (r) (Sneath & Sokal 1973). To optimize matching between pairs of traces, the program performed a secondary alignment of traces during calculation of correlation (Vauterin et al. 1991). Cluster analysis was achieved on the matrix of correlation values by the unweighted pair group method using arithmetic averages (UPGMA).

Results

The grouping of the protein electrophoregrams is summarized in Fig. 1 as a dendrogram. Sixteen clusters are delineated on the basis of qualitative and quantitative comparison of the protein profiles and literature data (see below). One of these clusters (I) groups all the investigated strains of the genus *Mrakia*. Each of the fifteen remaining clusters (II to XVI) groups strains of only one species with intraspecific correlation levels $r \geq 0.84$ (Fig. 1). Visual comparison of normalized electrophoregrams is presented in Fig. 2. Confidence can be given to the SDS-PAGE data because a good reproducibility ($r \geq 0.94$) was found between the protein patterns derived from duplicated cultures (data not shown).

Three species, *Cystofilobasidium bisporidii*, *C. capitatum* and *C. infirmominiatum*, formerly classified in the genus *Rhodosporidium* (Fell & Statzell Tallman 1984a) were transferred to the genus *Cystofilobasidium* on the basis of differences in morphological and chemotaxonomic characteristics (Oberwinkler et al. 1983; Hamamoto et al. 1988). Figure 1 demonstrates that three strains of each species group in a single cluster. The interspecific similarity between *C. capitatum* (cluster II) and *C. infirmominiatum* (cluster III) is $r = 0.87$. *C. bisporidii* (cluster IV) shows a similarity $r = 0.72$ with the other two species.

The genus *Filobasidium* contains three species:

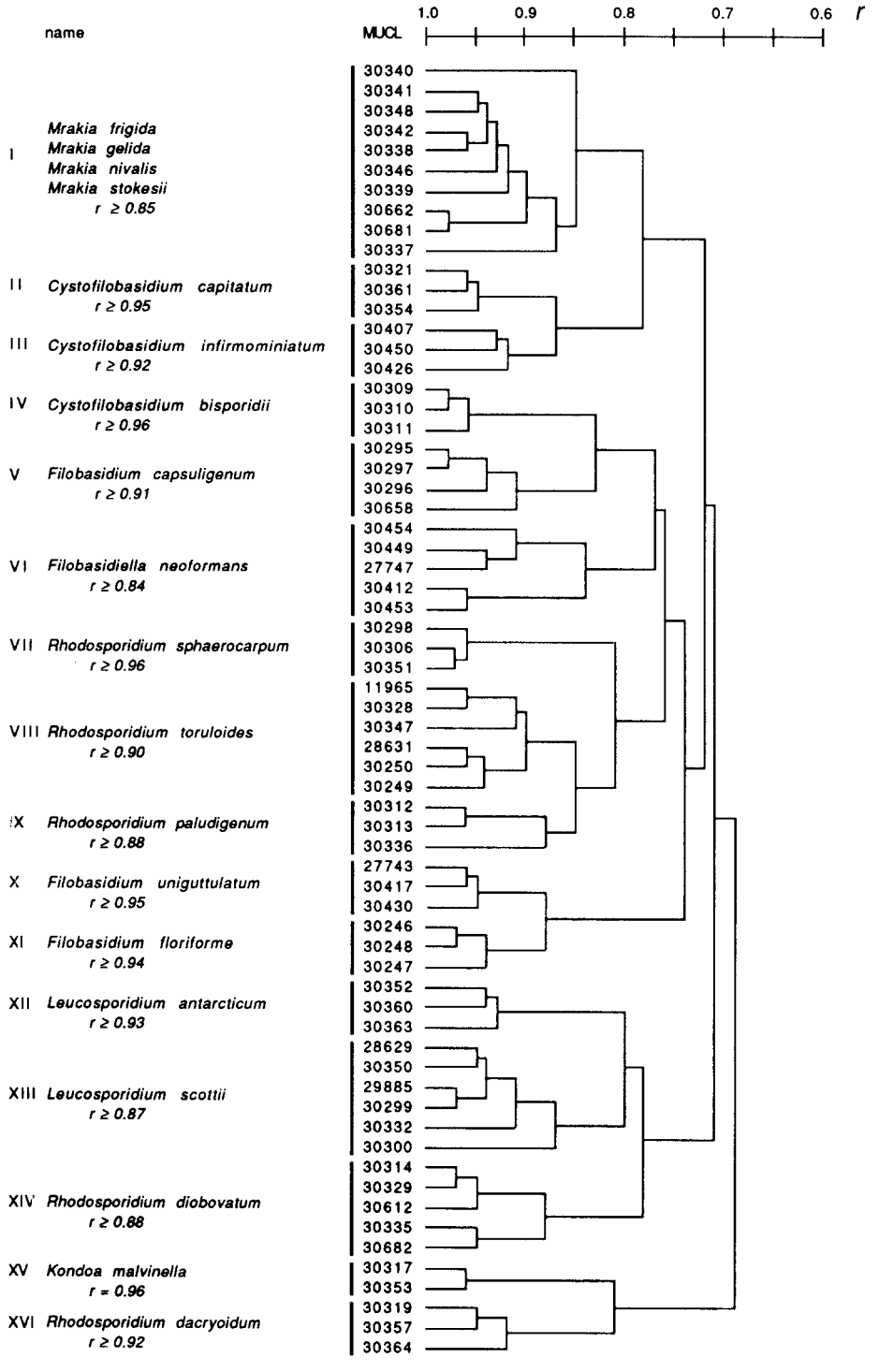

Fig. 1. Dendrogram showing the protein electrophoretic relationships between 65 basidiomycetous yeast strains. The correlation coefficient *r* was used and clustering was achieved by the UPGMA method. Roman numerals indicate cluster numbers.

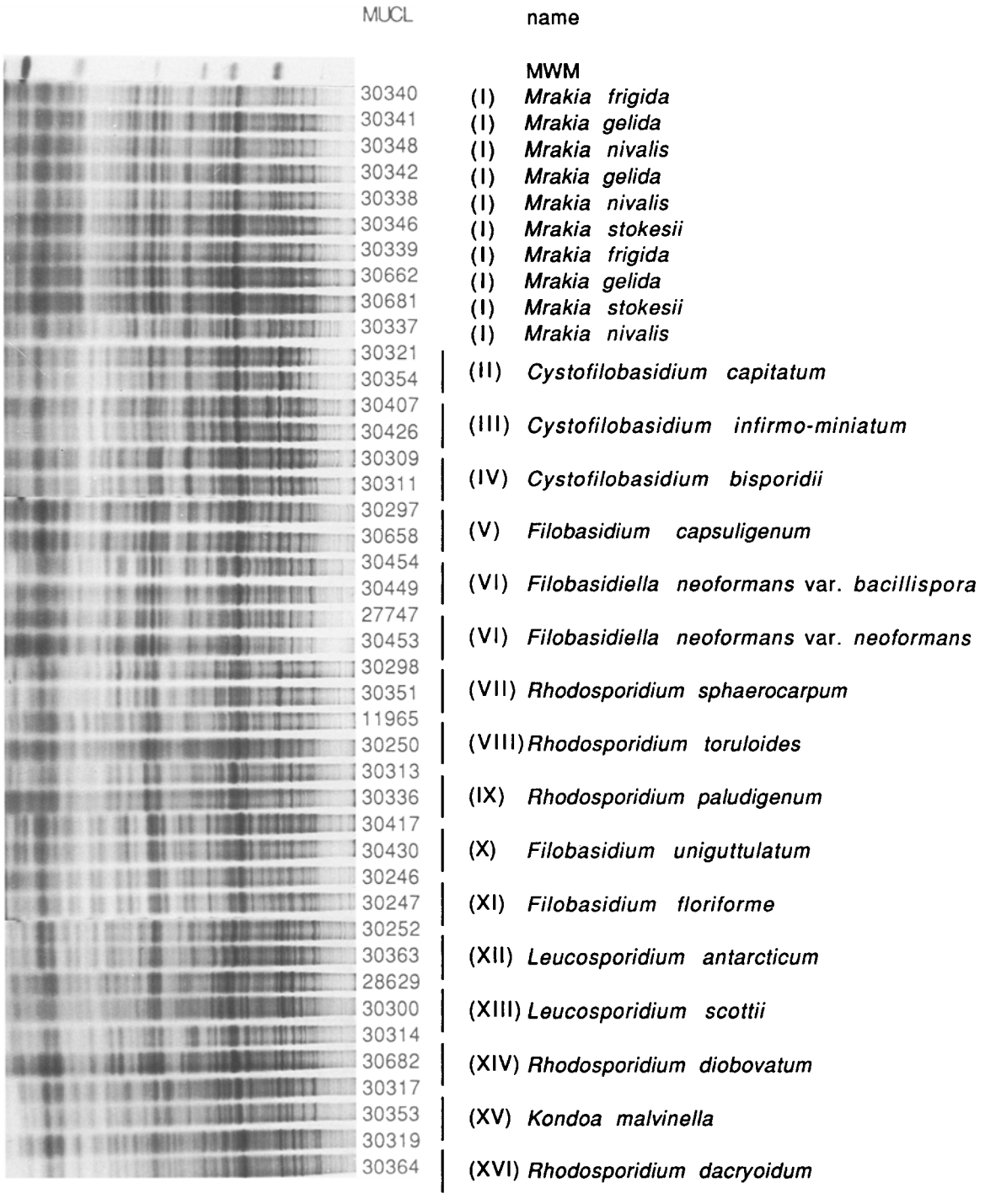

Fig. 2. Normalized electrophoregrams of whole-cell proteins from ten strains of species from the genus *Mrakia* and two strains of the other yeast species or varieties studied. The positions of the molecular weight markers (track labeled MWM) are indicated from left to right: lysosyme, 14.500; trypsin inhibitor, 20.100; carbonic anhydrase, 29.000; glyceraldehyde-3-phosphate dehydrogenase, 36.000; egg albumin, 45.000; bovine albumin, 66.000; β-galactosidase, 116.000. Roman numerals indicate cluster numbers.

F. capsuligenum, F. floriforme and *F. uniguttulatum* (Kwon-Chung & Fell 1984a). High intraspecific similarities are observed for four strains of *F. capsuligenum* (cluster V), three strains of *F. floriforme* (Cluster XI) and three strains of *F. uniguttulatum* (cluster X) (Fig. 1). *F. floriforme* and *F. uniguttulatum* have an interspecific correlation level $r = 0.88$. *F. capsuligenum* is electrophoretically more separated ($r = 0.74$) from the other two species of the genus.

The genus *Filobasidiella* contains one species with two varieties: *F. neoformans* var. *bacillispora* and *F. neoformans* var. *neoformans* (Kwon-Chung & Fell 1984b). Protein electrophoregrams of five strains of this species are visually highly similar (Fig. 2) and group at $r \geq 0.84$ (cluster VI, Fig. 1).

The genus *Kondoa* has been introduced with one species namely *K. malvinella* (Yamada et al. 1989) which was originally classified in the genus *Rhodosporidium* (Fell & Statzell Tallman 1984a). Two strains of this species form a single cluster (cluster XV).

The genus *Leucosporidium* was introduced by Fell and Statzell Tallman (1984b) as a yeast genus with six species. Four species, *L. frigidum, L. gelidum, L. nivale* and *L. stokesii*, are now classified in a separate genus *Mrakia* based on differences in morphological and chemotaxonomic characteristics (Yamada & Komagata 1987). From the two species remaining in the genus *Leucosporidium*, protein fingerprints of three strains from *L. antarcticum* (cluster XII) and six strains from *L. scottii* (cluster XIII) are compared and indicate that both species are well-characterized. The interspecific similarity of both species is $r = 0.80$.

As mentioned above, the genus *Mrakia* has been introduced with four species: *M. frigida, M. gelida, M. nivalis* and *M. stokesii* (Yamada & Komagata 1987). Figures 1 and 2 demonstrate that these species are electrophoretically highly similar (cluster I, $r \geq 0.85$) and that the intraspecific similarities are lower than the correlation levels found between some strains of different *Mrakia* species. For instance two *M. stokesii* strains have a similarity $r = 0.90$. One of these strains, the type strain (MUCL 30681), has a similarity $r = 0.98$ with the type strain of *M. gelida* (MUCL 30662).

Nine species were formerly recognized in the genus *Rhodosporidium* (Fell & Statzell Tallman 1984a). Three of the nine species, *R. bisporidii, R. capitatum* and *R. infirmominiatum* were transferred to the genus *Cystofilobasidium* and one species, *R. malvinellum*, was transferred to the genus *Kondoa* (see above). Based on protein electrophoresis, the remaining five species within the genus *Rhodosporidium* are differentiated into five separated clusters, corresponding to *R. dacryoidum* (cluster XVI), *R. diobovatum* (cluster XIV), *R. paludigenum* (cluster IX), *R. sphaerocarpum* (cluster VII) and *R. toruloides* (cluster VIII) (Fig. 1). The interspecific correlation between *R. toruloides* and *R. paludigenum* is $r = 0.85$. The *R. sphaerocarpum* strains are linked to these two species at a similarity $r = 0.81$. *R. diobovatum* and *R. dacryoidum* are more distantly related to the other *Rhodosporidium* species ($r = 0.71$ and $r = 0.69$, respectively).

Discussion

Intraspecific comparison

Except for the genus *Mrakia*, the protein fingerprinting technique confirmed species delimitation as stated by morphological structures and phenotypical features (Kreger-van Rij 1984). All species studied of the genera *Cystofilobasidium, Filobasidium, Filobasidiella, Kondoa, Leucosporidium* and *Rhodosporidium* showed a characteristic whole-cell protein fingerprint. For the basidiomycetous yeasts studied, the similarity values (r) for species delineation vary between 0.84 and 0.96. Also for delineation of ascomycetous yeast species correlation levels were usually higher than $r = 0.85$ (Vancanneyt et al. 1991). These results indicate that the expected similarity index for yeast conspecificity could be higher than approximately $r = 0.85$. Visual comparison of protein electrophoregrams demonstrates that intraspecific variability is usually due to quantitative rather than to qualitative differences. Changing the conditions of computation, e.g. by comparing a different part of the interpolated trace or by varying the number of

strains in the numerical analysis, can cause minor changes in similarity indices, but the groupings obtained at a level of 0.85 or higher will not be changed (results not shown). The results demonstrate that SDS-PAGE can be a valuable tool to characterize yeast species in addition to generally accepted criteria as interfertility tests and DNA-DNA homology studies.

Genetic relatedness of *Filobasidiella* strains was studied by Aulakh et al. (1981). Hybridizations of DNAs yielded relatedness values of only 55.2 to 63%, indicating that the DNAs are significantly different. They concluded that two species, *F. neoformans* and *F. bacillispora*, were closely related but distinct because of their ecological, epidemiological, genetic and biochemical differences. Kwon-Chung et al. (1982) confirmed interfertility between both species and transferred them to varieties of a single species. Protein electrophoregrams cannot differentiate these varieties, neither visually (Fig. 2) nor by numerical analysis ($r \geq 0.84$, cluster VI, Fig. 1).

Hamamoto et al. (1987) reported a study of DNA-DNA reassociations in the genus *Rhodosporidium* and found rather low intraspecific reassociation values within some species. Mating-type strains of *R. toruloides* showed 30–82% homology. In our study, six *R. toruloides* strains grouped together with a high similarity ($r \geq 0.90$, cluster VIII). Two of these strains (MUCL 30249 and MUCL 30250), sharing only 32% DNA-DNA homology (Hamamoto et al. 1987), displayed highly similar protein electrophoregrams ($r = 0.94$).

Strains of *R. diobovatum* varied in DNA-DNA reassociation values from 75 to 95% (Hamamoto et al. 1987). For strains of this species, similarities in protein electrophoregrams ranged from $r = 0.88$ to 0.99 (cluster XIV).

DNA-DNA homology between strains of *R. sphaerocarpum* was 64 to 99% (Hamamoto et al. 1987), whereas similarities in protein electrophoregrams were $r \geq 0.96$ (cluster VII).

Interspecific comparison

A number of criteria are considered useful for interspecific differentiation e.g. coenzyme Q types, DNA base composition and complex features such as the structure and chemical composition of the cell wall (Sugiyama et al. 1985). Numerical analyses of the protein electrophoregrams normally do not permit valid deductions concerning the genotypic relatedness between electrophoretic clusters (Kersters, 1985). Usually the similarity between strains of different species is lower than $r = 0.85$. For some species of the genera *Filobasidium* and *Cystofilobasidium* (see below) the similarity values were higher. Visual comparison (Fig. 2) clearly differentiates these species because of qualitative differences in protein profiles.

The results showed a high interspecific similarity ($r = 0.88$) between *Filobasidium floriforme* (cluster XI) and *F. uniguttulatum* (cluster X). Both species can be differentiated because of a higher intraspecific similarity within both taxa ($r \geq 0.94$, Fig. 1). In addition, qualitative differences are observed between the electrophoregrams of both clusters (Fig. 2). It was demonstrated that both species are differentiated by a coenzyme Q10 and Q9 system, respectively (Sugiyama et al. 1985; Yamada & Kondo 1976). *Filobasidium capsuligenum* (cluster V), possesses also a coenzyme Q10 system (Yamada & Kondo 1976) and was electrophoretically more distantly related.

Two species of the genus *Cystofilobasidium, C. capitatum* (cluster II) and *C. infirmominiatum* (cluster III), showed a similarity $r = 0.87$. These two species could be characterized individually by SDS-PAGE: the intraspecific similarities were $r \geq 0.92$ (Fig. 1) and considerable qualitative differences were found between protein patterns of both taxa (Fig. 2). *C. capitatum* and *C. infirmominiatum* have a coenzyme Q8 system, but differ considerably in DNA base composition: 59.2 and 66.3–67.5 mol % G + C, respectively (Hamamoto et al. 1986).

In this study, all *Mrakia* species grouped in a single cluster (I). Fell and Statzell Tallman (1984b) reported that among the four *Mrakia* species the assimilation of melibiose is different: positive in *M. nivalis* and *M. gelida* and negative in *M. frigida* and *M. stokesii*. In addition, *M. nivalis* is morphologically distinct from *M. frigida* by the absence of a

perfect metabasidium. Yamada and Matsumoto (1988) mentioned that all strains of *M. frigida* and *M. nivalis* examined had similarity values of 71% or more by an electrophoretic comparison of various enzymes. The same similarity values were obtained among the strains examined of *M. gelida* and *M. stokesii*. From these results, the authors accepted only two species in the genus *Mrakia*: *M. frigida* and *M. gelida*. *M. nivalis* and *M. stokesii* were considered to be synonyms of *M. frigida* and *M. gelida*, respectively. In Fig. 1 and 2 all *Mrakia* strains display qualitatively uniform protein patterns with similarities $r \geq 0.85$. Because the interspecific correlation levels are higher than the observed intraspecific similarities, data suggest that all *Mrakia* species may be synonymous. This seems to be confirmed by complete homology in the partial sequences of the positions 1451 to 1618 of the 18S rRNA in the type strains of the four *Mrakia* species (Yamada & Kawasaki 1989). Base differences in these positions are typical for differentiation between other basidiomycetous yeast species (Yamada & Kawasaki 1989; Yamada & Nakagawa 1990; Yamada et al. 1989). DNA-DNA homology and interfertility tests are required to measure the degree of relationship between the *Mrakia* species.

Conclusions

For the systematics of basidiomycetous yeasts, SDS-PAGE of cellular proteins is a valuable tool that characterizes all species investigated at the species level, except those belonging to the genus *Mrakia*. The four *Mrakia* species may be synonymous because of their highly similar electrophoretic protein patterns.

For identification of new isolates, the SDS-PAGE technique can easily be used as a routine procedure once a database of reference protein electrophoregrams has been constructed.

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