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Analysis of phylogenetic relationships among Ascomycota with yeast phases using ribosomal DNA sequences and cell wall sugars

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Abstract

Analysis of the monosaccharide composition of purified cell walls of unicellular and filamentous ascomycetous fungi shows three patterns: (1) the mannose glucose type (for most hemiascomycetous yeasts) (2) the mannose glucose galactose type (for several members of all three main ascomycetous clades) and (3) the mannose glucose galactose rhamnose type (for members of the Euascomycetes and the Protomyces/Schizosaccharomyces group).
In order to estimate the usefulness of the carbohydrate patterns for phylogenetic analysis we compared them with a phylogenetic tree based on 18SrRNA-gene sequences using the Neighbor-Joining Method. In contrast with the situation for basidiomycetous fungi, the Ascomycota show no fixed cell wall type for the three classes. Based on cell wall carbohydrates, sequence data and molecular characters the Hemiascomycetes appear as the first branch within the Ascomycota. A second clade, comprising the genera *Schizosaccharomyces*, *Pneumocystis*, *Taphrina*, *Protomyces*, *Neolecta* and *Saitoella*, appears as a sister group of the Euascomycetes. We discuss the erection of a new class for this group of ascomycetous fungi for which we propose the name Protomycetes.

Key words: Ascomycota, yeasts, cell wall carbohydrates, Protomycetes, phylogenetics

Introduction

The yeasts do not form a monophyletic group of organisms, but instead belong to different classes of the higher fungi (Eumycota). During the recent years we used the monosaccharide pattern of purified cell walls in addition with fermentative ability, urease production, diazonium blue B reaction, and the production of extracellular amyloid substances to determine the phylogenetic position of basidiomycetous yeasts (Dörfler 1990; Prillinger et al. 1990a, b, 1991a, b, 1993; Messner et al. 1994; Prillinger et al. 2001). Three different spectra of cell wall sugars have been found: (1) the Microbotryum type with mannose as the main sugar and fucose as the differentiation sugar, (2) the Ustilago type with glucose as the main sugar and mannose and galactose as additional sugars, and (3) the Tremella type with commonly dominant amounts of glucose and mannose as main sugars and xylose as differentiation sugar (small traces of galactose may be present too, as has been shown in the case of *Coniophora puteana* by O'Brien & Ralph (1966). These three types correspond well to the three basidiomycetous classes erected by Swann & Taylor (1995) upon comparisons of the small subunit RNA sequences, namely the Urediniomycetes, Ustilaginomycetes, and Hymenomycetes.

The scope of the present work was to determine the usefulness of the monosaccharide pattern of purified yeast cell walls for the delimitation of ascomycetous classes. For this purpose we analysed members of the "classic yeasts" (Saccharomycetales, Hemiascomycetes), yeast stages of the Euascomycetes, and species belonging to the so-called "Archiascomycetes" sensu Sugiyama & Nishida (1995). In this paper we present the results of this cell wall analysis and compare them with an evolutionary tree which we constructed using the sequences of the small ribosomal RNA of 96 fungal species.

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Table 1. Strains studied, their cell wall monosaccharide composition (in %), formation of extracellular amyloid compounds (EAC), hydrolysis of urea by urease, and ubiquinone system (UBI)

Species	Strain	Glu	Man	Gal	Rha	Fuc	EAC	Urease	UBI
HEMIASCOMYCETES									
Glu-Man-pattern									
Ambrosiozyma monospora (Saito) van der Walt	CBS 2554T	55	45				–	–	Q-7 (Yamada et al. 1976b)
Brettanomyces nanus (Smith et al.) Boekhout et al.	CBS 1945T	50	50				–	–	Q-9 (Yamada et al. 1980)
Candida albicans (Robin) Berkhout	HA671T	55	45				–	–	Q-9 (Yamada & Kondo 1972a)
Candida coipomensis Ramírez & Gonzàlez	HA1044T	56	44				–	–	n.d.
Candida ernobii (Lodder & Kreger-van Rij) Meyer	HA159T	57	43				–	–	Q-8 (Yamada & Kondo 1972a)
Candida glabrata (Anderson) Meyer & Yarrow	HA1039T	41	59				–	–	Q-6 (Yamada & Kondo 1972a)
Candida haemulonii (van Uden & Kolipinski) Meyer & Yarrow	HA153T	50	50				–	–	Q-9 (Yamada & Kondo 1972a)
Candida intermedia (Ciferri & Ashford) Langeron & Guerra	HA410T	34	66				–	–	Q-9 (Yamada & Kondo 1972a)
Candida maltosa Komagata et al.	HA1084T	54	46				–	–	Q-9 (Yamada & Kondo 1972a)
Candida nemodendra (van der Walt et al.) Meyer & Yarrow	HA158T	41	59				–	–	Q-7 (Lee & Komagata 1980)
Candida shehatae var. *shehatae* Buckley & van Uden	HA1049T	45	55				–	–	Q-9 (Yamada & Kondo 1972a)
Candida tenuis Diddens & Lodder	HA353	61	39				–	–	Q-9 (Yamada & Kondo 1972a)
Candida tropicalis (Castellani) Berkhout	HA1085T	51	49				–	–	Q-9 (Yamada & Kondo 1972a)
Citeromyces matritensis Santa María	CBS 2764T	49	51				–	–	Q-8 (Yamada et al. 1973a)
Clavispora lusitaniae Rodrigues de Miranda	HA774	75	25				–	–	Q-8 (Yamada & Kondo 1973)
Debaryomyces etchellsii (Kreger-van Rij) Maeda et al.	HA70T	73	27				–	–	Q-9 (Yamada et al. 1973a)
Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij	HA413T	66	34				–	–	Q-9 (Nakase & Suzuki 1985)
Debaryomyces maramus di Menna	HA1053T	71	29				–	–	Q-9 (Yamada et al. 1976b)
Debaryomyces occidentalis (Klöcker) Kurtzman & Robnett	CBS 819T	46	54				–	–	Q-9 (Yamada et al. 1977)
Debaryomyces robertsiae (van der Walt) Kurtzman & Robnett	HA988T	58	42				–	–	Q-9 (Yamada et al. 1973a)
Dekkera bruxellensis van der Walt	RBF620T	65	35				–	–	Q-9 (Yamada et al. 1980)
Eremothecium ashbyi Guilliermond	HA89	78	22				–	–	Q-6 (Yamada et al. 1987)
Eremothecium coryli (Peglion) Kurtzman	HA99T	47	53				–	–	Q-5 (Yamada et al. 1981)
Eremothecium gossypii (Ashby & Nowell) Kurtzman	HA88	60	40				–	–	Q-7 (Yamada et al. 1987)
Eremothecium sinecaudum (Holley) Kurtzman	HA661T	37	63				–	–	Q-9 (Yamada 1986)
Hanseniaspora uvarum (Niehaus) Shehata et al.	CBS 104	37	63				–	–	Q-6 (Yamada et al. 1976b)
Issatchenkia orientalis Kurtzman et al.	HA892T	42	58				–	–	Q-7 (Yamada et al. 1973b)
Kluyveromyces lactis (Dombrowski) van der Walt	HA61T	53	47				–	–	Q-6 (Yamada et al. 1976b)
Kluyveromyces polysporus van der Walt	HA65T	43	57				–	–	Q-6 (Prillinger et al. 1990a)
Lodderomyces elongisporus (Recca & Mrak) van der Walt	HA977	60	40				–	–	Q-9 (Yamada et al.1977)
Metschnikowia bicuspidata (Metschnikoff) Kamenski	HA672T	69	31				–	–	Q-9 (Yamada et al.1977)
Pachysolen tannophilus Boidin & Adzet	RBF215T	39	61				–	–	Q-8 (Yamada et al. 1973a)
Pichia anomala (Hansen) Kurtzman	HA1048T	52	48				–	–	Q-7 (Yamada et al. 1973a)
Pichia capsulata (Wickerham) Kurtzman	HA66T	34	66				–	–	Q-8 (Yamada et al. 1973a)
Pichia ciferrii (Lodder) Kurtzman	HA19	47	53				–	–	Q-7 (Yamada et al. 1973a)
Pichia farinosa (Lindner) Hansen	HA67	48	52				–	–	n.d.
Pichia haplophila Shifrine & Phaff	HA26T	70	30				–	–	Q-9 (Yamada et al. 1973a)
Pichia membranifaciens Hansen	HA895T	42	58				–	–	Q-7 (Yamada et al. 1973a)
Pichia minuta (Wickerham) Kurtzman	HA27	54	46				–	–	Q-7 (Yamada et al. 1973a)
Pichia pastoris (Guilliermond) Phaff	HA71T	25	75				–	–	Q-8 (Yamada et al. 1973a)

Pichia pijperi van der Walt & Tscheuschner	HA69T	36	64			–	–	Q-7 (Yamada et al. 1973a)
Pichia pini (Holst) Phaff	HA68T	43	57			–	–	Q-7 (Yamada et al. 1973a)
Pichia quercuum Phaff & Knapp	HA72T	35	65			–	–	Q-7 (Yamada et al. 1973a)
Saccharomyces cerevisiae Meyen ex Hansen	HA227T	42	58			–	–	Q-6 (Prillinger et al. 1990a)
Saccharomycodes ludwigii Hansen	HA982	37	63			–	–	Q-6 (Yamada et al. 1976a)
Saccharomycopsis capsularis Schiönning	HA1089T	54	46			–	–	Q-8 (Yamada et al. 1976b)
Saccharomycopsis fibuligera (Lindner) Klöcker	HA105	51	49			–	–	Q-8 (Yamada et al. 1976b)
Saccharomycopsis javanensis (Klöcker) Kurtzman & Robnett	RBF617T	63	37			–	–	Q-8 (Yamada et al. 1976b)
Wickerhamia fluorescens Soneda	HA148T	53	47			–	–	Q-9 (Yamada et al. 1976a)
Williopsis saturnus var. *mrakii* (Wickerham) Kurtzman	HA3T	47	53			–	–	Q-7 (Yamada et al. 1973a)
Zygosaccharomyces rouxii (Boutroux) Yarrow	HA989T	44	56			–	–	Q-6 (Yamada et al. 1976b)
Glu-Man-Gal pattern								
Arxula adeninivorans (Middelhoven et al.) van der Walt et al.	HA1092T	61	30	9		+?	–	Q-9 (van der Walt et al. 1990)
Candida apis var. *apis* (Lavie ex van Uden & Vidal-Leiria) Meyer & Yarrow	HA877	63	29	8		–	–	Q-9 (Suzuki et al. 1999)
Candida bertae var. *bertae* Ramírez & González	HA1054T	53	36	11		–	–	n.d.
Candida blankii Buckley & van Uden	HA878T	51	39	10		–	–	n.d.
Candida castrensis Ramírez & González	HA1052T	33	38	29		–	–	n.d.
Candida etchellsii (Lodder & Kreger-van Rij) Meyer & Yarrow	HA876T	62	28	10		–	–	Q-9 (Yamada & Kondo 1972a)
Candida gropengiesserii (Harrison) Meyer & Yarrow	HA414T	47	43	10		–	–	Q-9 (Yamada & Kondo 1972a)
Candida paludigena Golubev & Blagodatskaya	HA1100T	38	56	6		–	–	n.d.
Candida sorbophila (Nakase) Meyer & Yarrow	HA874T	28	45	27		–	–	Q-9 (this paper)
Candida valdiviana Grinbergs & Yarrow	HA1042T	45	49	6		–	–	n.d.
Candida versatilis (Etchells & Bell) Meyer & Yarrow	HA875T	58	18	24		–	–	Q-9 (Yamada & Kondo 1972a)
Debaryomyces tamarii Ohara & Nonomura	HA411T	47	28	25		+?	–	n.d.
Dipodascus albidus de Lagerheim	HA1093	47	40	13		–	–	n.d.
Dipodascus capitatus de Hoog et al.	HA106	37	42	21		–	–	Q-9 (Yamada et al. 1982)
Geotrichum fermentans (Diddens & Lodder) von Arx	HA108	37	42	21		–	–	n.d.
Lipomyces lipofer Lodder & Kreger-van Rij ex Slooff	HA101T	53	41	6		+	–	Q-10 (Yamada et al. 1986)
Lipomyces starkeyi Lodder & Kreger-van Rij	RBF625T	53	30	17		+	–	Q-9 (Yamada et al. 1986)
Nadsonia commutata Golubev	HA658T	59	36	5		–	–	Q-6 (Yamada et al. 1992)
Nadsonia fulvescens (Nadson & Konokotina) Sydow	HA32	32	50	18		–	–	Q-6 (Yamada et al. 1976a)
Pichia ofunaensis (Makiguchi & Asai) Kurtzman	HA1098T	37	49	14		–	–	n.d.
Pichia tannicola Jacob	HA1097T	65	27	8		–	–	n.d.
Schizoblastosporion starkeyi-henrici Ciferri	HA11T	50	32	7		–	–	Q-6 (Yamada et al. 1987)
Stephanoascus ciferrii Smith et al.	HA1090T	53	45	2		–	–	Q-9 (Yamada & Smith 1985)
Stephanoascus smithiae Giménez-Jurado	HA1043T	53	37	10		–	–	Q-9 (Giménez-Jurado et al. 1994)
Wickerhamiella domercqiae van der Walt & Liebenberg	HA987T	28	52	20		–	–	Q-9 (Prillinger et al. 1990a)
Yarrowia lipolytica (Wickerham et al.) van der Walt & von Arx	HA123T	44	42	14		–	–	Q-9 (Yamada & Kondo 1972b)
PROTOMYCETES								
Taphrinales								
Protomyces inouyei Hennings	ATCC16175	74	14	1	11	+	+	Q-10 (Yamada et al. 1983)
Protomyces pachydermus von Thümen	HA242	58	22	2	18	+	+	Q-10 (Yamada et al. 1983)
Taphrina deformans (Berk.) Tulasne	HA839	79	12	6	3	+	+	Q-10 (Prillinger et al. 1990a)
Taphrina padi (Jacz.) Mix	HA100	62	21	11	6	+	+	n.d.
Taphrina polystichii Mix	HA844	67	19	1	12	+	+	Q-10 (Prillinger et al. 1990a)

Table 1. (Continued).

Species	Strain	Glu	Man	Gal	Rha	Fuc	EAC	Urease	UBI
Taphrina populina Fries	HA845	77	12	4	5		+	+	Q-10 (Prillinger et al. 1990a)
Taphrina pruni (Fuck.) Tulasne	RBF688	62	21	11	4		+	+	n.d.
Taphrina vestergrenii Giesenhagen	HA244	64	20	1	7	7	+	+	Q-10 (Prillinger et al. 1990a)
Schizosaccharomycetales									
Schizosaccharomyces octosporus Beijerinck	HA978	90	6	3			–	+	Q-9 (Prillinger et al. 1990a)
Schizosaccharomyces pombe Lindner	HA983	76	14	8			–	+	Q-10 (Yamada et al. 1973b)
Protomycete of uncertain phylogenetic position									
Saitoella complicata Goto et al.	HA102T	70	23	7			–	+	Q-10 (Ahearn et al. 1998)
EUASCOMYCETES									
Ascosphaerales									
Eremascus albus Eidam	MA914	77	9	14			–	+	n.d.
Chaetothyriales									
Capronia parasitica (Ellis & Everhart) Müller et al.	HA394T	27	49	24			–	+	Q-9 (this paper)
Exophiala pisciphila McGinnis & Ajello	HA397T	84	13	3			–	+	Q-10 H2 (this paper)
Dothideales									
Aureobasidium pullulans (de Bary) Arnaud	HA392T	83	12	5			+	+	Q-10 H2 (Yamada et al. 1989)
Dothiora rhamni-alpinae Froidevaux	HA396T	79	16	5			–	+	Q-10 H2 (Yamada et al. 1989)
Pringsheimia chamaecyparidis Froidevaux	HA459T	76	11	13			n.d.	n.d.	Q-10 H2 (Yamada et al. 1989)
Sydowia polyspora (Brefeld & von Travel) Müller	HA393T	83	13	4			–	+	Q-10 H2 (Yamada et al. 1989)
Hypocreales									
Verticillium dahliae Klebahn	MD141	72	11	17			–	+	Q-10 H2 (this paper)
Onygenales									
Blastomyces dermatitidis Gilchrist & Stokes	HA151	39	28	33			+?	+	n.d.
Ophiostomatales									
Ophiostoma bicolor Davidson & Wells	HA103	51	28	5	16		–	+	Q-10 H2 (this paper)
Ophiostoma ips (Rumbold) Nannfeldt	HA96	51	26	3	19		–	+	Q-10 H2 (this paper)
Ophiostoma quercus (Georgevitch) Nannfeldt	HA207	62	19	3	16		–	+	n.d.
Ophiostoma ulmi (Buisman) Nannfeldt	HA97	48	28	4	20		–	+	Q-10 H2 (this paper)
Sporothrix albicans Saksena	HA163	39	33	2	26		–	+	Q-10 H2 (this paper)
Sporothrix schenckii Hektoen & Perkins	HA653T	58	34	2	6		–	+	Q-10 H2 (Suzuki & Nakase 1986)
Euascomycetes of uncertain phylogenetic positions									
Acremonium strictum Gams	HA1022	81	9	10	0.6		+?	+	n.d.
Calyptrozyma arxii Boekhout & Spaay	HA1083	62	23	14	1		–	+	Q-10 (Boekhout et al. 1995)
Hyphozyma lignicola Hutchison et al.	HA679T	47	23	12	18		–	+	Q-10 (this paper)
Hyphozyma roseonigra de Hoog & Smith	HA680T	30	26		44		–	+	Q-10 H2 (this paper)
Hyphozyma sanguinea (Ramírez) de Hoog & Smith	HA682T	46	22	5	27		–	+	Q-10 (this paper)
Hyphozyma variabilis var. *odora* de Hoog & Smith	HA683T	32	27	3	38		–	+	Q-10 (this paper)
Hyphozyma variabilis var. *variabilis* de Hoog & Smith	HA685T	46	23	2	29		–	+	Q-10 (this paper)
Lecythophora hoffmannii (van Beyma) Gams	HA550	79	13	4	4		+	+	Q-10 H2 (this paper)
Lecythophora lignicola (van Beyma) Gams	HA551	64	22	5	9		+	+	Q-10 H2 (this paper)
Symbiotaphrina buchneri Gräbner ex Gams & von Arx	HA1058	49	37	14	0.5		–	+	Q-9 (12%) Q-10 (88%) (Moore & Flinn 1991)
Symbiotaphrina kochii Jurzitza ex Gams & von Arx	HA1059T	47	24	16	13		–	+	Q-9 (12%) Q-10 (88%) (Moore & Flinn 1991)

Materials and methods

Fungal strains

The fungal strains examined in this study and their cell wall monosaccharide composition are listed in Table 1. Strain numbers correspond to the following strain collections:

HA, MD: VIAM Culture Collection, Institute of Applied Microbiology, University of Agriculture, Vienna, Austria
CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
RBF: Raiffeisen Bioforschung, Zuckerforschungs-Institut, Tulln, Austria

Qualitative and quantitative monosaccharide pattern of purified yeast cell walls

The neutral sugar composition was determined after trifluoracetic acid hydrolysis as described in Lopandic et al. (1996).

Ubiquinone system

Ubiquinone analysis was performed as described in Messner et al. (1994). For the high-performance liquid chromatography (HPLC) analysis we used a Hewlett-Packard Series 1050 HPLC pump, a model 1050 diode array detector, and a model 1050 automatic sampler (Hewlett-Packard GmbH, Waldbronn, Germany)

Phenotypic characterisation

Formation of extracellular amyloid compounds (EAC) and hydrolysis of urea by urease were investigated according to the methods described by van der Walt and Yarrow (1984).

Sequence analysis

The sources for the 18SrRNA sequences of 96 fungal species are listed in Table 2. Editing, alignment and storage of sequences were carried out using the ClustalW program (Thompson et al. 1994). Alignment was performend on 1680 bases. Computation of trees was done using the programs DNADIST (Parameter "Kimura2"; Kimura 1980), FITCH, NEIGHBOR JOINING, SEQBOOT, and CONSENSE in the PHYLIP package. The basidiomycete clade was used as the outgroup. Bootstrap confidence values were calculated from 100 repeats.

Results

In the present work we determined the cell wall sugar composition of a number of ascomycetous true yeasts and unicellular stages of dimorphic or predominantly filamentous fungi. The unicellular stages of Euascomycetes belong to the orders Ascosphaerales (*Eremascus*), Chaetothryriales (*Capronia*, *Exophiala*), Dothideales (*Dothiora*, *Pringsheimia*, *Sydowia*), Hypocreales (*Verticillum*), Onygenales (*Blastomyces*), and Ophiostomatales (*Ophiostoma*, *Sporothrix*), and to some species of uncertain phylogenetic position (e.g. in *Acremonium*, *Calyptrozyma*, *Hyphozyma*, *Lecythophora*, *Symbiotaphrina*). For the recently described euascomycetous species *Calyptrozyma arxii* a new order was postulated by Boekhout et al. (1995).

The qualitative and quantitative monosaccharide patterns of purified cell walls for 114 ascomycetous fungal strains are shown in Table 1.

Three different monosaccharide patterns were found:
(1) the mannose glucose type
(2) the glucose mannose galactose type
(3) the glucose mannose galactose rhamnose type.

Fucose and xylose, which are present as additional sugars in some basidiomycetous strains (Microbotryum type, Tremella type), are totally lacking in the Ascomycota investigated so far, with the exception of *Taphrina vestergrenii* (Taphrinales) which also shows small amounts of fucose (7%) besides glucose (64%), mannose (20%), galactose (1%), and rhamnose (7%).

The mannose glucose type was found in 51 species within the Hemiascomycetes, with members of the following genera: *Ambrosiozyma*, *Brettanomyces*, *Candida*, *Citeromyces*, *Clavispora*, *Debaryomyces*, *Dekkera*, *Eremothecium*, *Hanseniaspora*, *Issatchenkia*, *Kluyveromyces*, *Lodderomyces*, *Metschnikowia*, *Pachysolen*, *Pichia*, *Saccharomyces*, *Saccharomycodes*, *Saccharomycopsis*, *Wickerhamia*, *Williopsis*, *Zygosaccharomyces*. The proportion of mannose was in the range from 22% (*Eremothecium ashbyi*) to 75% (*Pichia pastoris*). No general prevalence of either mannose or galactose was observed within any genus with several strains analysed (e.g. *Candida*, *Debaryomyces*, *Pichia*).

No member of the Schizosaccharomyces/Protomyces group and no Euascomycete so far analysed shows this mannose glucose carbohydrate pattern.

Cell walls containing glucose, mannose and galactose were found in 26 strains belonging to the Hemiascomycetes within the genera *Arxula*, *Candida*, *Debaryomyces*, *Dipodascus*, *Geotrichum*, *Lipomyces*, *Nadsonia*, *Pichia*, *Schizoblastosporion*, *Stephanoascus*, *Wickerhamiella*, and *Yarrowia*. The proportion of glucose ranged from 28% (*C. sorbophila* and *W. domercqiae*) to 65% (*P. tannicola*), the proportion of mannose from 18% (*C. versatilis*) to 56% (*C. paludigena*). In all strains but one (*C. versatilis*) galactose was the least common carbohydrate, ranging from 2% (*S. ciferrii*) to 27% (*C. sorbophila*).

The same glucose mannose galactose pattern was found also in members of the "Protomyces-clade",

Table 2. 18SrRNA-gene sequences used in this study for the Neighbor-Joining tree construction.

Species	Gen bank accession number	Reference
Alternaria alternata	U05194	Morales et al. (1995)
Alternaria brassicae	U05196	Jasalavich et al. (1995)
Ascosphaera apis	M83264	Berbee & Taylor (1992)
Aspergillus flavus	X78537	Melchers et al. (1994)
Aureobasidium pullulans	M55639	Illingworth et al. (1991)
Auricularia polytricha	L22255	Swann & Taylor (1993)
Blastomyces dermatitidis	M55624	Bruns et al. (1992)
Blumeria graminis	AB033476	Mori et al., unpublished
Boletus satanas	M94337	Bruns et al. (1992)
Botryosphaeria ribis	U42477	Berbee (1996)
Candida albicans	X53497	Hendriks et al. (1989)
Candida bertae var. *bertae*	AB018155	Suzuki et al. (1999)
Candida blankii	AB018125	Suzuki et al (1999)
Candida edax	AB018129	Suzuki et al. (1999)
Candida glabrata	X51831	Wong & Clark-Walker (1990)
Candida maltosa	D14593	Ohkuma et al. (1993)
Candida tropicalis	M55527	Hendriks et al. (1991)
Candida valdiviana	AB015910	Suzuki et al. (1999)
Capronia mansonii	X79318	Haase et al. (1995)
Ceratocystis fimbriata	U43777	Issakainen et al. (1997)
Clavispora lusitaniae	M55526	Hendriks et al. (1991)
Cochliobolus sativus	U42479	Berbee (1996)
Cryphonectria parasitica	L42441	Chen et al., unpublished
Cucurbitaria elongata	U42482	Berbee (1996)
Cystofilobasidium capitatum	D12801	Suh & Sugiyama (1993)
Debaryomyces hansenii	X58053	Hendriks et al. (1992)
Dekkera bruxellensis	X58052	Hendriks et al. (1992)
Dekkera custersiana	X83817	Cai et al. (1996)
Dekkera naardensis	X85110	Cai et al. (1996)
Dipodascopsis uninucleata	U00969	Berbee & Taylor (1993)
Dipodascus albidus	X69840	Wilmotte et al. (1993)
Dothidea insculpta	U42474	Berbee (1996)
Eremascus albus	M83258	Berbee & Taylor (1992)
Eremothecium sinecaudum	U53443	Prillinger et al. (1997)
Exophiala dermatitidis	X79312	Haase et al. (1995)
Filobasidiella neoformans	X60183	Van de Peer et al. (1992)
Galactomyces geotrichum	X69842	Wilmotte et al. (1993)
Gibberella pulicaris	AF081467	O'Donnell, unpublished
Hanseniaspora uvarum	X69844	Wilmotte et al. (1993)
Helvella lacunosa	U53378	Landvik et al. (1997)
Histoplasma capsulatum	Z75306	Okeke et al., unpublished
Issatchenkia orientalis	M55528	Hendriks et al. (1991)
Kluyveromyces lactis	X51830	Maleszka & Clark-Walker (1990)
Kluyveromyces polysporus	X69845	Wilmotte et al. (1993)
Leptosphaeria maculans	U04238	Morales et al. (1995)
Leucostoma persooni	M83259	Berbee & Taylor (1992)
Lipomyces lipofer	X69848	Wilmotte et al. (1993)
Meliola juddiana	AF021793	Saenz & Taylor (1999)
Metschnikowia bicuspidata	X69842	Wilmotte et al. (1993)
Microascus cirrosus	M89994	Berbee & Taylor (1992)
Microbotryum violaceum	U77062	Swann & Taylor (1995)
Mixia osmundae	D14163	Nishida et al. (1995)
Morchella esculenta	U42642	O'Donnell et al. (1996)
Mrakia frigida	D12802	Suh & Sugiyama (1993)
Mycosphaerella mycopappi	U43449	Dong et al., unpublished
Nectria aureofulva	AB013010	Ogawa et al., unpublished

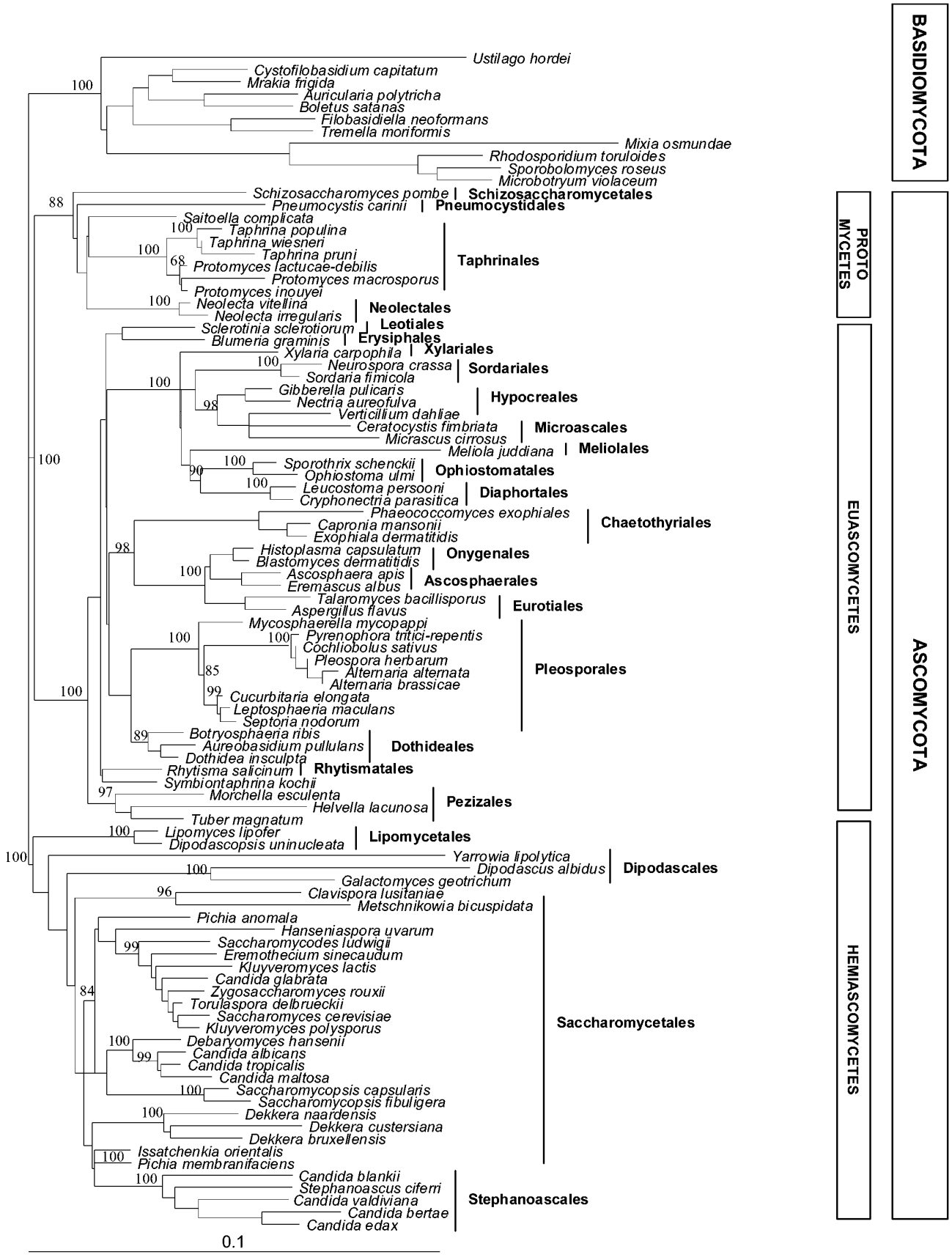
Table 2. (Continued).

Species	Gen bank accession number	Reference
Neolecta irregularis	Z47721	Landvik (1996)
Neolecta vitellina	Z27393	Landvik et al. (1993)
Neurospora crassa	X04971	Sogin et al. (1986)
Ophiostoma ulmi	M83261	Berbee & Taylor (1992)
Phaeococcomyces exophiales	X80709	Haase et al. (1995)
Pichia anomala	X58054	Hendriks et al. (1992)
Pichia membranifaciens	X58055	Hendriks et al. (1992)
Pleospora herbarum	U43458	Dong et al., unpublished
Pneumocystis carinii	X12708	Edman et al. (1988)
Protomyces inouyei	D11377	Nishida et al. (1993)
Protomyces lactucae-debilis	D14164	Nishida & Sugiyama (1994)
Protomyces macrosporus	D85143	Nishida et al., unpublished
Pyrenophora tritici-repentis	U42486	Berbee (1996)
Rhodosporidium toruloides	X60180	Suh & Sugiyama (1993)
Rhytisma salicinum	U53370	Landvik, unpublished
Saccharomyces cerevisiae	J01353	Mankin et al. (1986)
Saccharomycodes ludwigii	X69843	Wilmotte et al. (1993)
Saccharomycopsis capsularis	X69847	Wilmotte et al. (1993)
Saccharomycopsis fibuligera	X69841	Wilmotte et al. (1993)
Saitoella complicata	D12530	Nishida & Sugiyama (1993)
Schizosaccharomyces pombe	Z19578	Lapeyre & Feliu, unpublished
Sclerotinia sclerotiorum	L37541	Gargas & Taylor (1995)
Septoria nodorum	U04236	Morales et al. (1995)
Sordaria fimicola	X69851	Wilmotte et al. (1993)
Sporobolomyces roseus	X60181	Van de Peer et al. (1992)
Sporothrix schenckii	M85053	Berbee & Taylor (1992)
Stephanoascus ciferrii	AB018141	Suzuki et al. (1999)
Symbiotaphrina kochii	D49656	Noda & Kodama (1996)
Talaromyces bacillisporus	D14409	Berbee et al. (1995)
Taphrina populina	D14165	Nishida & Sugiyama (1994)
Taphrina pruni	AB000956	Sjamsuridzal et al. (1997)
Taphrina wiesnerii	D12531	Nishida & Sugiyama (1993)
Torulaspora delbrueckii	X53496	Hendriks et al. (1990)
Tremella moriformis	U00977	Berbee & Taylor (1993)
Tuber magnatum	AF054901	Percudani et al. (1999)
Ustilago hordei	U00973	Berbee & Taylor (1993)
Verticillium dahliae	U33637	Messner et al. (1996)
Xylaria carpophila	Z49785	Andersson, unpublished
Yarrowia lipolytica	M60312	Barns et al. (1991)
Zygosaccharomyces rouxii	X58057	Hendriks et al. (1992)

namely *Schizosaccharomyces octosporus*, *Sch. pombe* and *Saitoella complicata*. Apart from these unicellular organisms we found this cell wall type within the filamentous or dimorphic euascomycetous genera *Aureobasidium*, *Blastomyces*, *Capronia*, *Dothiora*, *Eremascus*, *Exophiala*, *Pringsheimia*, *Sydowia*, and *Verticillium*.

The third monosaccharide type, which contains glucose, mannose, galactose and rhamnose is spread within the Euascomycetes, namely the Ophiostomatales (*Ophiostoma* sp., *Sporothrix* sp.) and in different genera of uncertain phylogenetic positions *(Hyphozyma*, *Lecythophora*, *Symbiotaphrina*). The dimorphic, plant-pathogenic genera *Taphrina* and *Protomyces*, belonging to the Protomyces/Schizosaccharomyces group, contain also these four cell wall sugars. In contrast, no member of the Hemiascomycetes analysed so far was found to contain rhamnose or fucose in its cell walls.

In order to clarify the evolutionary significance of the carbohydrate pattern for ascomycetous true yeasts and filamentous fungi with unicellular stages, we constructed an evolutionary tree using the small subunit sequences of 96 fungal species. Two of the sequences, namely that of the plant pathogens *Verticillium dahliae* (Hypocreales) and *Eremothecium sinecaudum* (syn.


Ustilago hordei
Cystofilobasidium capitatum
Mrakia frigida
Auricularia polytricha
Boletus satanas
Filobasidiella neoformans
Tremella moriformis
Mixia osmundae
Rhodosporidium toruloides
Sporobolomyces roseus
Microbotryum violaceum
Schizosaccharomyces pombe | Schizosaccharomycetales
Pneumocystis carinii | Pneumocystidales
Saitoella complicata
Taphrina populina
Taphrina wiesneri
Taphrina pruni
Protomyces lactucae-debilis
Protomyces macrosporus
Taphrinales
Protomyces inouyei
Neolecta vitellina
Neolecta irregularis | Neolectales
Sclerotinia sclerotiorum | Leotiales
Blumeria graminis | Erysiphales
Xylaria carpophila | Xylariales
Neurospora crassa
Sordaria fimicola
Sordariales
Gibberella pulicaris
Nectria aureofulva
Hypocreales
Verticillium dahliae
Ceratocystis fimbriata
Micrascus cirrosus
Microascales
Meliola juddiana | Meliolales
Sporothrix schenckii
Ophiostoma ulmi
Ophiostomatales
Leucostoma persooni
Cryphonectria parasitica
Diaphortales
Phaeococcomyces exophiales
Capronia mansonii
Exophiala dermatitidis
Chaetothyriales
Histoplasma capsulatum
Blastomyces dermatitidis
Onygenales
Ascosphaera apis
Eremascus albus
Ascosphaerales
Talaromyces bacillisporus
Aspergillus flavus
Eurotiales
Mycosphaerella mycopappi
Pyrenophora tritici-repentis
Cochliobolus sativus
Pleospora herbarum
Alternaria alternata
Alternaria brassicae
Pleosporales
Cucurbitaria elongata
Leptosphaeria maculans
Septoria nodorum
Botryosphaeria ribis
Aureobasidium pullulans
Dothidea insculpta
Dothideales
Rhytisma salicinum | Rhytismatales
Symbiontaphrina kochii
Morchella esculenta
Helvella lacunosa
Pezizales
Tuber magnatum
Lipomyces lipofer
Dipodascopsis uninucleata | Lipomycetales
Yarrowia lipolytica
Dipodascus albidus
Dipodascales
Galactomyces geotrichum
Clavispora lusitaniae
Metschnikowia bicuspidata
Pichia anomala
Hanseniaspora uvarum
Saccharomycodes ludwigii
Eremothecium sinecaudum
Kluyveromyces lactis
Candida glabrata
Zygosaccharomyces rouxii
Torulaspora delbrueckii
Saccharomyces cerevisiae
Kluyveromyces polysporus
Debaryomyces hansenii
Candida albicans
Candida tropicalis
Candida maltosa
Saccharomycopsis capsularis
Saccharomycopsis fibuligera
Dekkera naardensis
Dekkera custersiana
Dekkera bruxellensis
Saccharomycetales
Issatchenkia orientalis
Pichia membranifaciens
Candida blankii
Stephanoascus ciferri
Candida valdiviana
Candida bertae
Candida edax
Stephanoascales
0.1
BASIDIOMYCOTA
PROTO MYCETES
EUASCOMYCETES
HEMIASCOMYCETES
ASCOMYCOTA

Holleya sinecauda, Saccharomycetales), have been obtained by our group (Messner et al. 1996, Prillinger et al. 1997), the other sequences were derived from GenBank. In order to document the Schizosaccharomyces/Protomyces group as completely as possible we added the genera *Neolecta* and *Pneumocystis*, which do not contain yeast stages. In the few cases where no full-length 18SrDNA sequences were available for the species analysed chemotypically, sequences were used from other members of the respective genera. For example, *Exophiala pisciphila* (Diaphortales) was used for the cell wall analysis, but for the phylogenetic tree construction only a sequence from *E. dermatitidis* was available. The congeneric status of the two species has been confirmed by Uijthof & de Hoog (1995). The same situation applies to *Capronia parasitica* and *C. mansonii* (Diaphortales).

The resulting Neighbor-Joining tree (Fig. 1) shows an unequivocal, deep division between the ascomycetous and the basidiomycetous fungi. The Ascomycota are subdivided into three clades which may be assigned taxonomically to three different classes: the first branch consists of the "true yeasts" or Hemiascomycetes; the second clade – which we are here naming "Protomycetes" – consists of a rather heterogeneous group of unicellular yeasts (*Schizosaccharomyces*, *Saitoella*), dimorphic plant pathogens (*Taphrina*, *Protomyces*), filamentous fungi (*Neolecta*), and the human pathogen *Pneumocystis carinii*; and finally the third clade comprises the Euascomycetes itself with mainly filamentous and dimorphic species. The yeastlike symbiont of anobiid beetles, *Symbiotaphrina kochii,* also falls in this latter group.

The topology of our phylogenetic tree is supported by high bootstrap values for the main branches (estimated with 100 cycles), namely the first branch leading to the Hemiascomycetes, and the two sister branches leading to the Protomycetes and the Euascomycetes. Using the zygomycete *Mucor racemosus* as the outgroup (instead of the basidiomycete clade) does not change the internal resolution of the three ascomycetous branches (data not shown).

The test on hydrolysis of urea by urease was negative for all hemiascomycetous strains (Table 1), whereas this reaction was positive for all strains belonging to the "Protomycetes-group" and the Euascomycetes.

Extracellular amyloid compounds (EAC) were produced within the Hemiascomycetes only by strains belonging to the Lipomycetales (*L. lipofer*, *L. starkeyi*), a weak colouring was observed also by *A. adenivorans* and *D. tamarii* (both Dipodascales). Within the "Protomyces-group" the genera *Protomyces* (*P. inouyei*, *P. pachydermus*) and *Taphrina* (*T. deformans*, *T. padi*, *T. polystichii*, *T. populina*, *T. pruni*, *T. vestergrenii*) show a positive reaction, whereas the Schizosaccharomycetales (*Sch. octosporus*, *Sch. pombe*) were negative. EAC was produced within the Euascomycetes by *L. hoffmannii* and *L. lignicola* (uncertain systematic position), and by *A. pullulans* (Dothideales), but not by the other members of the Dothideales analysed so far (*D. rhamni-alpinae*, *S. polyspora*). Weak positive reactions were found for *B. dermatitidis* (Onygenales) and *A. strictum* (uncertain systematic position), no extracellular compounds were produced by the members of the Ascosphaerales (*E. albidus*), Chaetothyriales (*C. parasitica*, *E. pisciphila*), Hypocreales (*V. dahliae*), Ophiostomatales (genera *Ophiostoma* and *Sporothrix*), nor within the genera *Calyptrozyma*, *Hyphozyma* and *Symbiotaphrina*.

The number of isoprene units of the ubiquinone system (coenzyme Q) was analysed in this study for several strains belonging to the Euascomycetes (Table 1). In most cases coenzyme Q-10 and Q-10 H2 was found, e.g. within *E. pisciphila* (Chaetothyriales), *V. dahliae* (Hypocreales), *O. bicolor*, *O. ips*, *O. ulmi*, *S. albicans* (all Ophiostomatales), and *Hyphozyma* and *Lecythophora*. Coenzyme Q-9 was detected within *C. parasitica* (Chaetothyriales).

Discussion

The composition of the fungal cell walls has been used frequently for studying the systematics and taxonomy of filamentous fungi (Bartnicki-Garcia 1968, Leal et al. 1997, Leal & Bernabé 1998), yeasts (Gorin & Spencer 1970), and lichen mycobionts (Teixeira et al. 1995). In a broader phylogenetic approach, the usefulness of the monosaccharide pattern of purified cell walls for the delimitation of classes has been shown recently for basidiomycetous fungi (Prillinger et al. 1990a, b, 1991a, b, 1993; Messner et al. 1994). The three cell wall types correspond to the three basidiomycetous classes erected by Swann & Taylor (1995) on phylogenetic information from the small subunit ribosomal genes. The Microbotryum type corresponds to the Urediniomycetes clade, the Ustilago type to the Ustilaginomycetes clade, and the Tremella type to the Hymenomycetes clade.

Fig. 1. Unrooted Neighbor-Joining tree of 96 fungal species on the basis of 1680 bases of the 18SrRNA-gene. In addition to yeasts and yeast-like fungi, also taxa consisting of solely filamentous ascomycetous fungi were integrated to document the Ascomycota as extensively as possible. The robustness of the tree was assessed by bootstrap analysis using 100 repeats. Only values greater than 65% are given above the branches. The Basidiomycota clade was used as the outgroup. Scale bar indicates accumulated changes per 100 nucleotides.

In this study we determined the composition of the cell walls from 114 ascomycetous strains, most of them true yeasts and unicellular stages of dimorphic or predominantly filamentous fungi (Table 1). Similar to the situation with basidiomycetous fungi, also the ascomycetous fungi show three different monosaccharide patterns:
(1) the mannose glucose type
(2) the glucose mannose galactose type
(3) the glucose mannose galactose rhamnose type.

In our analyses the mannose glucose type was found exclusively within the Hemiascomycetes, in species belonging to 21 different genera (*Ambrosiozyma*, *Brettanomyces*, *Candida*, *Citeromyces*, *Clavispora*, *Debaryomyces*, *Dekkera*, *Eremothecium*, *Hanseniaspora*, *Issatchenkia*, *Kluyveromyces*, *Lodderomyces*, *Metschnikowia*, *Pachysolen*, *Pichia*, *Saccharomyces*, *Saccharomycodes*, *Saccharomycopsis*, *Wickerhamia*, *Williopsis*, *Zygosaccharomyces*).

In their study of the monosaccharide pattern of several Zygomycetes, Hoddinott & Olsen (1972) reported the occurrence of the glucose mannose type also for several members of the Entomophtorales, namely *Entomophtora coronata*, *E. exitialis*, *E. thaxteriana* and *E. virulenta*. *Conidiobolus stromoideus* (Entomopthorales) showed glucose only, *Basidiobolus ranarum* – recently reclassified within the Chytridiomycota (Tanabe et al. 2000, Prillinger et al. 2001) – gave glucose, galactose and fucose.

Cell walls containing glucose, mannose and galactose were found within 12 genera of the Hemiascomycetes (*Arxula*, *Candida*, *Debaryomyces*, *Dipodascus*, *Geotrichum*, *Lipomyces, Nadsonia*, *Pichia*, *Schizoblastosporion*, *Stephanoascus*, *Wickerhamiella*, *Yarrowia*).

The heterogeneity of the genus *Candida* as shown by Kurtzman & Robnett (1998) using partial sequences of the 26SrRNA gene is corroborated by our findings that some members of this genus (*Candida apis* var. *apis*, *C. bertae* var. *bertae, C. blankii*, *C. castrensis*, *C. etchellsii*, *C. gropengiesserii*, *C. paludigena*, *C. sorbophila*, *C. valdiviana*, *C. versatilis*) also belong to the glucose mannose galactose type. Suzuki et al. (1999) proposed the erection of the new order Stephanoascales for members of the Hemiascomycetes containing galactose in the cell walls. Several new anamorphic yeast species isolated from the hindgut of termites (Schweigkofler et al. 2000) and from mushrooms (Schweigkofler 1998) fall in the same group, as has been shown using cell wall analysis and sequencing of the whole 18SrRNA gene and of the D1/D2-domain of the 26SrRNA gene (Schweigkofler et al. 2000). The name *Galactocandida* was proposed for the anamorphic members of the order Stephanoascales.

The glucose mannose galactose pattern is not restricted to yeast species belonging to the Hemiascomycetes, but can be found also in some unicellular species of the "Protomyces-clade" (e.g. *Sch. pombe*) and several predominantly filamentous Euascomycetes belonging to the orders Ascosphaerales (*E. albidus*), Dothideales (*A. pullulans*, *D. rhamni-alpinae*, *P. chamaecyparidis*), Chaetothyriales (*C. parasitica*, *E. pisciphila*), Hypocreales (*V. dahliae*) and Onygenales (*B. dermatitidis*). The occurrence of this type in *Aspergillus*, *Fusarium*, *Gibberella*, *Myrothecium*, *Neurospora*, *Penicillium* and *Sesquicillium* was reported recently (Leal et al. 1997, Leal & Bernabé 1998, O. Ahrazem pers. comm.).

The glucose mannose galactose and rhamnose pattern was found within dimorphic species of the "Protomycetes-group" (*Taphrina* sp., *Protomyces* sp.), and in several Euascomycetes of the genera *Ophiostoma*, *Sporothrix*, *Hyphozyma*, *Lecythophora* and *Symbiotaphrina*. It was also reported from *Botrytis aclada* and *Monilinia fructigena* (Leotiales; Leal & Bernabé 1998). In contrast, *Xylaria hypoxylon* (Xylariales) shows a mannose glucose pattern as do most Hemiascomycetes, but with an extremely elevated percentage of glucose (95%) in comparison to mannose (5%) (O'Brien & Ralph 1966). In this case we believe that the presence of only two carbohydrates in the cell wall of this highly evolved euascomycete could be a reduced character, resulting from the loss of one or more cell wall sugars.

Our Neighbor-Joining tree (Fig. 1), based on analyses of the 18SrRNA-genes from 96 fungal strains, divides the Ascomycota into three classes: the Hemiascomycetes (mainly unicellular "true yeasts"), the Protomycetes (unicellular, dimorphic, and filamentous species), and the large group of the Euascomycetes with predominantly filamentous (and several dimorphic) species. The yeast-like *Symbiotaphrina kochii*, living symbiotically within beetles, clusters also within the Euascomycetes. This result corroborates the theory of Noda & Kodama (1996), who proposed that this unicellular organism with no known sexual stage may have had a filamentous euascomycetous ancestor and then may have lost the ability to produce mycelia after taking residence in the beetles. A close affinity to the family Taphrinaceae, which has been postulated based on morphological criteria (Gams & von Arx 1980, von Arx 1981), therefore seems rather unlikely. The topology of the Neighbor-Joining tree confirms our earlier Maximum-Likelihood analysis of 63 species representing the three ascomycetous clades (Schweigkofler 1998). Basically the same phylogenetic trends were found also by Schweigkofler & Prillinger (1999) and Prillinger et al. (2001) when using Neighbor-Joining analysis with slightly different species samples.

Within the Hemiascomycetes Kurtzman & Fell (1998) accept only a single order, Saccharomycetales (Endomycetales). As discussed in detail by Prillinger et al. (2001) we propose four different orders based on the qualitative and quantitative monosaccharide patterns of

purified yeast cell walls and complete 18S rDNA sequences (Fig. 1.; Suzuki et al. 1999): Saccharomycetales, Dipodascales, Lipomycetales and Stephanoascales. Whereas the Saccharomycetales and Lipomycetales can be delimited by the cell wall monosaccharide pattern and the presence or absence of extracellular amyloid compounds (Saccharomycetales: glucose, mannose, EAS: –; Lipomycetales: glucose, mannose, galactose, EAS: +), it is not possible to separate the Dipodascales and Stephanoascales by the cell wall monosaccharide pattern. Within both orders glucose, mannose and galactose dominate, but species with the glucose mannose pattern appear intermingled (Suzuki et al. 1999). Sequences of the complete 18S rRNA gene are important to decide whether a species belongs to Dipodascales or Stephanoascales.

In contrast to Kudryavtsev (1960), who proposed the name Saccharomycetales for strictly unicellular yeasts, our concept of the Saccharomycetales includes also the genus *Eremothecium* with dimorphic (*E. sinecaudum*, *E. coryli*) and filamentous (*E. gossypii*, *E. ashbyi*, *E. cimbalariae*) members. Prillinger et al. (1997) included these species into the Saccharomycetaceae based on sequence analysis of the genes coding for the 18SrRNA and ITS regions, and on analysis of cell wall sugars, Ubiquinone side chains and presence of dityrosine.

Nishida & Sugiyama (1994; see also Sugiyama & Nishida 1995), proposed a major new lineage within the Ascomycota based on Neighbor-Joining analysis of small subunit ribosomal genes, and named it Archiascomycetes because of some primitive characters and an early divergence from the other Ascomycota. Those authors included in this clade the species *Taphrina deformans*, *T. populina*, *T. wiesneri*, *Protomyces lactucae-debilis*, *P. inouyei*, *Schizosaccharomyces pombe*, *Saitoella complicata*, and *Pneumocystis carinii*, with the following common characters: a hyphal or yeast-like assimilative state, a sexual ascogenous state lacking ascogenous hyphae, the lack of any ascomata or conidiomata, and asexual budding or fission. The Archiascomycetes sensu Nishida & Sugiyama (1994) correspond largely to the class Taphrinomycetes erected by Cavalier-Smith (1987) on the basis of comparative ultrastructure, lysine biosynthesis, and 5S rRNA sequence data.

Interestingly, Landvik et al. (1993), presented evidence from sequence analysis that the filamentous, fruit-body producing ascomycete *Neolecta vitellina* clusters with members of Protomyces/Schizosaccharomyces type, and they erected the new order Neolectales for that species. The analysis (see Landvik 1996) of another member of that genus, *N. irregularis*, corroborates this close relationship to the Protomyces/Schizosaccharomyces group, which is in agreement with our results. The fruit-body of *Neolecta* is characterised by the absence of sterile hyphae between the asci, the lack of ascogenous hooks prior to ascus development, and the unusual combination of inoperculate asci having amyloid ascus walls (Redhead 1977). The existence of such fruit bodies within the Protomyces/Schizosaccharomyces group correlates with the close relationship of this clade to the Euascomycetes, as deduced by our molecular analysis.

Nishida & Sugiyama (1994) postulated a sister group relationship for the Hemiascomycetes and Euascomycetes, and that they evolved more recently than the "Archiascomycetes". Similar results were obtained by Berbee & Taylor (1993) who introduced the name "basal ascomycetes" for the "Archiascomycetes sensu Nishida and Sugiyama". However, both phylogenetic analyses show a rather low statistic support by bootstrap analysis.

Sequence analyses of the 5SrRNA gene of several ascomycetous species by Walker (1985) also suggest the existence of three branches within the Ascomycota, which correspond to our classes. Even if the branching arrangement is not fully resolved in his dendrogram, Walker (1985), using a present-day ancestor analysis, concluded that the Schizosaccharomyces/Protomyces group has a rather isolated position within the Ascomycota, with closer affinities to the Hemiascomycetes than to the Euascomycetes. In contrast to *Sch. pombe* whose sequence is slightly closer to the main ascomycete sequences than to the main basidiomycete sequences, the opposite was found by Walker (1985) for *Protomyces inundatus*. A similar "Basidiomycete-like" 5SrRNA type was observed for *Taphrina deformans* by Gottschalk & Blanz (1985). These findings corroborate the theory of Savile (1955) who considered the Protomycetales and Taphrinales to represent descendants of an early branch of the Ascomycota that gave rise to the Basidiomycota.

On the other hand, the phylogenetic tree proposed by Cai et al. (1996) corroborates our results: using the 18SrRNA genes of 82 ascomycetous and 24 basidiomycetous strains they obtained also three ascomycetous branches, with the Euascomycetes and the Protomyces/Schizosaccharomyces group as sister taxa and the Hemiascomycetes as a separate, early diverging branch.

Similar results were obtained by Kurtzman (1993) using partial sequences of 18S and 26SrRNA and calculation with the PAUP program: in his analysis, *Taphrina deformans* and *Protomyces inundatus* formed a sister group to the three fruit-body forming ascomycetes included in his study, namely *Eremascus fertilis*, *Emericella nidulans* and *Ceratocystis fimbriata*. These groups together in turn formed a sister group to the budding yeasts.

Eriksson et al. (1993), studying the molecular differences between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, performed a parsimony analysis of the 18SrRNA genes of six fungal species (*S. cerevi-*

siae, *Sch. pombe*, *Neolecta vitellina*, *Neurospora crassa*, *Leotia lubrica*, *Inermisia aggregata*) and three higher eukaryotes (*Arabidopsis thaliana*, *Artemia salina*, *Homo sapiens*), with the cellular slime mold *Dictyostelium discoideum* as outgroup. The resulting most parsimonious tree shows *Sch. pombe* as a sister group to the "higher" ascomycetes. In their analysis *Sch. pombe* was much closer to these ascomycetes than to *Saccharomyces*. Based on this sequence comparison and a large number of other molecular data, they corroborate the new order Schizosaccharomycetales introduced by Prillinger et al. (1990a) for unicellular fungi with cylindrical cells, which divide by fission and produce globose or ellipsoidal ascospores without fruiting bodies. The relationship of *Sch. pombe* to the genera *Taphrina* and *Protomyces* was not further analysed in their study.

The ability to hydrolyse urea is an important physiological marker in yeast taxonomy (Barnett et al. 1990), often used for fast and reliable discrimination between basidiomycetous yeasts, which give a positive reaction, and the hemiascomycetous yeasts, which lack this activity. In our analysis all tested Euascomycetes and the members of the Protomyces/Schizosaccharomyces clade show a positive urease test (Table 1, see also Laaser 1989), indicating a common enzymatic pathway.

As discussed in detail by Prillinger et al. (2001), the yeast form, denoted by the term "coccal" (i.e. a unicellular organism having a rigid cell wall outside its plasma membrane), occupies a basal position in the Zygo-, Asco-, and Basidiomycota (Oberwinkler, pers. comm.) but seems to be derived in the Chytridiomycota (e.g. *Basidiobolus*: Nagahama et al. 1995). A yeast/hyphae dimorphism is common in all classes of the Ascomycota and Basidiomycota, especially in primitive representatives. It seems to be fundamental for a rapid evolution of the fungi.

From an ecological point of view, most "true yeasts" belonging to the Hemiascomycetes are living saprotrophically, connected with a wide range of living and dead plants. Several *Candida* species, e.g. *C. albicans*, *C. dubliniensis* and *C. glabrata*, are well known human pathogens. The genus *Eremothecium*, as a member of the Saccharomycetaceae, includes plant-pathogenic filamentous (*E. ashbyi*, *E. gossypii*) and dimorphic species (*E. coryli*, *E. sinecaudum*) in warm zones. Dissemination of these fungi seems to be dependent on animal vectors, e.g. plant-feeding bugs (Heteroptera). *Lipomyces lipofer* is a typical saprotrophic soil yeast.

Yeast-like and dimorphic members of the Protomycetes comprise pathogens of wooden and herbaceous plants in temperate zones (*Taphrina*, *Protomyces*) as well as saprotrophic forms (*Schizosaccharomyces*). Nothing is known about the ecological function of *Saitoella complicata* which has been isolated only once from soil in Bhutan (Goto et al. 1987).

Human pathogens occur also in the dimorphic euascomycetous orders Onygenales (*Histoplasma capsulatum*, *Blastomyces dermatitidis*) and Ophiostomatales (*Sporothrix schenckii*). *Aureobasidium pullulans* (Dothideales) is a wide-spread saprotrophic inhabitant of the phyllosphere, *Ophiostoma ulmi* and the closely related *O. novo-ulmi* (Ophiotomatales) are the causal agents of the Dutch Elm disease.

Our concept of a basal position of the Hemiascomycetes when compared with the other Ascomycota (Euascomycetes and members of the Protomyces/Schizosaccharomyces clade) is supported by a number of important molecular and morphological characters (most data are available only for *S. cerevisiae* and *Sch. pombe*, since these are the species best analysed at the molecular level):

1) The nuclear genome size of the Hemiascomycetes appears to be about one third the size of *Aspergillus*, *Schizosaccharomyces* and the basidiomycetous yeasts (Kurtzman 1985, 1993). More recent data from the whole genome sequencing project, however, suggest that the genomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are similar in size: *S. cerevisiae* 13.4 Mb (Goffeau et al. 1996), *Sch. pombe* about 14 Mb (Hoheisel et al. 1993). The 14 megabasepairs are organized in 3 compact chromosomes in *Sch. pombe*, which resemble higher eukaryotes. In *S. cerevisiae* 16 rather primitive chromosomes (see items 2 and 5 below) were found.

2) *S. cerevisiae* shows a rather unique cell cycle with the doubling of the spindle pole body and the formation of a short mitotic spindle already in the S phase. The G2 phase is missing. On the other hand, the cell cycle of *Sch. pombe* resembles the higher eukaryotes with characteristic G1, S, G2, and mitose phases (Byers & Goetsch 1975).

3) *S. cerevisiae* has a very compact nuclear genome with very few (223) introns (Mewes et al. 1997a, b). The higher frequency of introns in the genes of *Sch. pombe* resembles the situation in the higher eukaryotes.

4) *S. cerevisiae* is one of few eukaryotes that can live without functional mitochondria. On the other hand, *Sch. pombe* requires mitochondria for survival as do the higher eukaryotes (among other eukaryotes lacking mitochondria are the microsporidia, highly specialised intracellular parasites which have been shown recently to be related to fungi; Hirt et al. 1999).

5) The centromers of *S. cerevisiae* are smaller and lack the repeated sequences typical for higher eukaryotes. *Sch. pombe* has centromers which resemble those in "higher" ascomycetes and have similar function (Clarke & Baum 1990).

6) The length of the mating type loci: *S. cerevisiae* has the shortest known idiomorphs for the mating type loci (a: 640 bp, α: 750 bp). The respective lengths for

Sch. pombe are P: 1.1 kb, M: 1.1 kb; for *Ustilago maydis* a1: 4.5 kb, a2: 8kb; for *Podospora pauciseta* mat+: 3.8 kb, mat-: 4.7kb, for *Neurospora crassa* A: 5.3 kb, a: 3.2 kb; and for the basidiomycetous yeast *Cryptococcus neoformans* mat α: 35–45 kb (the mat-a locus of *C. neoformans* is not yet determined; Moore & Edman 1993).

7) The lack of fruit bodies among the Hemiascomycetes could be interpreted as a primitive rather than reduced character. Within the Protomyces/Schizosaccharomyces group the Neolectales produce club-shaped fruit bodies, which are up to 7 cm tall and differ from other ascomycetous fruit bodies mainly by the absence of sterile hyphae (paraphyses) between the asci, the lack of ascogenous hooks (croziers) prior to ascus development, and the unusual combination of inoperculate asci having amyloid ascus walls (Redhead 1977)

8) The coenzyme Q of the Hemiascomycetes (Table 1) contains a variable number of isoprene units ranging from Q-5 to Q-9 (Q-10 was found in *Lipomyces lipofer*). On the other hand, most strains of the Protomyces/Schizosaccharomyces clade analysed so far contain coenzyme Q-10 (with the exception of *Schizosaccharomyces octosporus* which has Q-9), resembling the Euascomycetes, which contain in most cases coenzyme Q-10 and Q-10 (H2). Coenzyme Q-9 was found only rarely in some euascomycetous strains (e.g. *Capronia parasitica*, *Symbiotaphrina* spp.). No strain with less isoprene units has been found within the Euascomycetes so far. Basidiomycetous yeasts possess coenzyme Q systems with Q-7, Q-8, Q-9, Q-10 and Q-10 (H2) (Kurtzman & Fell 1998, and references therein).

9) The Hemiascomycetes include morphologically primitive fungi (within the genus *Eremothecium*) with coenocytic "siphonal" ontogenetic stages which resemble the Zygo- and Chytridiomycota.

The examples presented in this paper should underline the importance to use a broad polyphasic approach towards the analyses of fungal relationships, taking into consideration not only restricted sequence information from single genes, but in addition a variety of morphological, ultrastructural, molecular, and developmental characters.

References

Ahearn, D. G., Sugiyama, J. & Simmons, R. B. (1998): Description of anamorphic ascomycetous genera and species 70: *Saitoella*. Pp. 600–601 in: Kurtzman C. P. & Fell, J. W. (eds.) The Yeasts. A Taxonomic Study. Elsevier, Amsterdam.

Arx, J. A. von (1981): The Genera of Fungi Sporulating in Pure Culture. J. Cramer, Hirschberg, Poland.

Barnett, J. A., Payne, R. W. & Yarrow, D. (1990): Yeasts: Characteristics and Identification. Cambridge University Press, Cambridge, UK.

Barns, S. M., Lane, D. J., Sogin, M. L., Bibeau, C. & Weisburg, W. G. (1991): Evolutionary relationships among pathogenic *Candida* species and relatives. J. Bacteriol. 173: 2250–2255.

Bartnicki-Garcia, S. (1968): Cell wall chemistry, morphogenesis and taxonomy of fungi. Annu. Rev. Microbiol. 22: 273–285.

Berbee, M. L. (1996): Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol. Biol. Evol. 13: 462–470.

Berbee, M. L. & Taylor, J. W. (1992): Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequences. Mol. Biol. Evol. 9: 278–284.

Berbee, M. L. & Taylor, J. W. (1993): Dating the evolutionary radiations of the true fungi. Can. J. Bot. 71: 1114–1127.

Berbee, M. L., Yoshimura, A., Sugiyama, J. & Taylor J. W. (1995): Is *Penicillium* monophyletic? An evolution in the family Trichocomaceae from 18S, 5.8S, and ITS ribosomal DNA sequence data. Mycologia 87: 210–222.

Boekhout, T., Roeijmans, H. & Spaay, F. (1995): A new pleomorphic ascomycete, *Calyptrozyma arxii* gen. et sp. nov., isolated from the human lower oesophagus. Mycol. Res. 99: 1239–1246.

Bruns, T. D., Vilgalys, R., Barns, S. M., Gonzalez, D., Hibbett, D. S., Lane, D. J., Simon, L., Stickel, S., Szaro, T. M., Weisburg, W. G. & Sogin, M. L. (1992): Evolutionary relationships within the Fungi: analyses of nuclear small subunit rRNA sequences. Mol. Phylogenet. Evol. 1: 231–241.

Byers, B. & Goetsch, L. (1975): Electronic microscopic observations on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae*. Proc. Natl Acad. Sci. 72: 5056–5069.

Cai, J., Roberts, I. N. & Collins, M. D. (1996): Phylogenetic relationships among members of the ascomycetous yeast genera *Brettanomyces*, *Debaryomyces*, *Dekkera,* and *Kluyveromyces* deduced by small-subunit rRNA gene sequences. Int. J. Syst. Bacteriol. 46: 542–549.

Cavalier-Smith, T. (1987): The origin of fungi and pseudofungi. Pp. 1–15 in: Rayner, A. D. M., Brasier, C. M. & Moore, D. (eds.) Evolutionary Biology of the Fungi. Cambridge University Press, Cambridge, UK.

Clarke, L. & Baum, M. P. (1990): Functional analysis of a centromere from fission yeast: a role for centromere-specific repeated DNA sequences. Mol. Cell. Biol. 10: 1863–1872.

Dörfler, C. (1990): Vergleichende Untersuchungen zum biochemischen Aufbau der Zellwand an Hefestadien von niederen und höheren Basidiomyceten. Bibl. Mycologica 129: 1–164.

Edman, J. C., Kovacs, J. C., Masur, H., Santi, D. V., Elwood, H. J. & Sogin, M. L. (1988): Ribosomal RNA sequences show *Pneumocystis carinii* to be a member of the fungi. Nature 334: 519–522.

Eriksson, O. E., Svedskog, A. & Landvik, S. (1993): Molecular evidence for the evolutionary hiatus between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Syst. Ascomycetum 11: 119–162.

Gams, W. & Arx, J. A. von (1980): Validation of *Symbiotaphrina* (imperfect yeasts). Persoonia 10: 542–543.

Gargas, A. & Taylor, J. W. (1995): Phylogeny of Discomycetes and early radiations of the apothecial Ascomycotina in-

ferred from SSU rDNA sequence data. Exp. Mycol. 19: 7–15.

Giménez-Jurado, G., Cidadao, A. J. & Beijn-van der Waaij, A. (1994): A novel heterothallic ascomycetous yeast species: *Stephanoascus smithiae,* teleomorph of *Candida edax*. Syst. Appl. Microbiol. 17: 237–246.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H. & Oliver, S. G. (1996): Life with 6000 genes. Science 274: 546–567.

Gorin, P. A. J. & Spencer, J. F. T. (1970): Proton magnetic resonance spectroscopy – an aid in identification and chemotaxonomy of yeasts. Adv. Appl. Microbiol. 13: 25–89.

Goto, S., Sugiyama, J., Hamamoto, M. & Komagata, K. (1987): *Saitoella*, a new anamorph genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formerly identified as *Rhodotorula glutinis*. J. Gen. Appl. Microbiol. 33: 75–85.

Gottschalk, M. & Blanz, P. A. (1985): Untersuchungen an 5S ribosomalen Ribonukleinsäuren als Beitrag zur Klärung von Systematik und Phylogenie der Basidiomyceten. Z. Mykol. 51: 205–243.

Haase, G., Sonntag, L., Peer, Y. van de, Uijthof, J. M. J., Podbielski, A. & Melzer-Krick, B. (1995): Phylogenetic analysis of ten black yeast species using nuclear small subunit rRNA gene sequences. Antonie van Leeuwenhoek 68: 19–33.

Hendriks, L., Goris, A., De Bruyn, K. & De Wachter, R. (1990): The nucleotide sequence of the small ribosomal subunit RNA of the yeast *Torulaspora delbrueckii*. Nucleic Acids Res. 18 (15): 4611.

Hendriks, L., Goris, A., Peer, Y. van de, Hennebert, G. L. & De Wachter, R. (1989): The nucleotide sequence of the small ribosomal subunit RNA of the yeast *Candida albicans* and the evolutionary position of the fungi among the eukaryotes. Syst. Appl. Microbiol. 12: 223–229.

Hendriks, L., Goris, A., Peer, Y. van de, Neefs, J. M., Vancanneyt, M., Kersters, K., Berny, J. F., Hennebert, G. L. & De Wachter, R. (1992): Phylogenetic relationships among ascomycetes and ascomycete-like yeasts as deduced from small ribosomal subunit RNA sequences. Syst. Appl. Microbiol. 15: 98–104.

Hendriks, L., Goris, A., Peer, Y. van de, Neefs, J. M., Vancanneyt, M., Kersters, K., Hennebert, G. L. & De Wachter, R. (1991): Phylogenetic analysis of five medically important *Candida* species as deduced on the basis of small ribosomal subunit RNA sequences. J. Gen. Microbiol. 137: 1223–1230.

Hirt, R. P., Logsdon, J. M., Healy, B., Dorey, M. W., Doolittle, W. F. & Embley, T. M. (1999): Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc. Natl Acad. Sci. 96: 580–585.

Hoddinott, J. & Olsen, O. (1972): A study of the carbohydrates in the cell walls of some species of the Entomophtorales. Can. J. Bot. 50: 1675–1679.

Hoheisel, J. D., Maier, E., Mott, R., McCarthy, L., Gregoriev, A. V., Schalkwyk, L. C., Nizetic, D., Francis, F. & Lehrach, H. (1993): High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast *S. pombe*. Cell 73: 109–120.

Illingworth, C. A., Andrews, J. H., Bibeau, C. & Sogin, M. L. (1991): Phylogenetic placement of *Athelia bombacina*, *Aureobasidium pullulans* and *Colletotrichum gloeosporioides* inferred from sequence comparisons of small-subunit ribosomal RNAs. Exp. Mycol. 15: 65–75.

Issakainen, J., Jalava, J., Eerola, E. & Cambell, C. K. (1997): Relatedness of *Pseudallescheria*, *Scedosporium* and *Graphium* pro parte based on SSU rDNA sequences. J. Med. Vet. Mycol. 35: 389–398.

Jasalavich, C. A., Morales, V. M., Pelcher, L. E. & Seguin-Swartz, G. (1995): Comparison of nuclear ribosomal DNA sequences from *Alternaria* species pathogenic to crucifers. Mycol. Res. 99: 604–614.

Kimura, M. (1980): A simple method for estimating evolutionary rate of base substitutions throughout comparative studies of nucleotide sequences. J. Mol. Evol. 2: 87–90.

Kudryavtsev, V. I. (1960). Die Systematik der Hefen. Akademie Verlag, Berlin.

Kurtzman, C. P. (1985): Molecular taxonomy of the fungi. Pp. 35–63 in: Benett, J. W. & Lasure, L. L. (eds.) Gene Manipulations in Fungi. Academic Press, Orlando, Florida.

Kurtzman, C. P. (1993): Systematics of the ascomycetous yeasts assessed from ribosomal RNA sequence divergence. Antonie van Leeuwenhoek 63: 165–174.

Kurtzman, C. P. & Fell, J. W. (1998): The Yeasts: a Taxonomic Study, ed. 4, revised. Elsevier, Amsterdam.

Kurtzman, C. P. & Robnett, C. J. (1998): Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331–371.

Laaser, G. (1989): Vergleichende systematische Studien an Basidiomycetenhefen unter besonderer Berücksichtigung der Hefestadien. Biblioth. Mycol. 130: 1–325.

Landvik, S. (1996): *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA sequences. Mycol. Res. 100: 199–202.

Landvik, S., Egger, K. N. & Schumacher, T. (1997): Towards a subordinal classification of the Pezizales (Ascomycota): phylogenetic analyses of SSU rDNA sequences. Nord. J. Bot. 17: 403–418.

Landvik, S., Eriksson, O. E., Gargas, A. & Gustafsson, P. (1993): Relationship of the genus *Neolecta* (Neolectales ordo nov., Ascomycotina) inferred from 18SrDNA sequences. Systema Ascomycetum 11: 107–118.

Leal, J. A. & Bernabé, M. (1998): Taxonomic applications of polysaccharides. Pp. 153–181 in: Frisvad, J. C., Bridge, P. D. & Arora, D. K.(eds.): Chemical Fungal Taxonomy. M. Dekker, Inc. New York.

Leal, J. A., Gomez-Miranda, B., Prieto, A., Domenech, J., Ahrazem, O. & Bernabé, M. (1997): Possible chemotypes from cell wall polysaccharides, as an aid in the systematics of *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Mycol. Res. 101: 1259–1264.

Lee, J.-D. & Komagata, K. (1980): Taxonomic study of methanol-assimilating yeasts. J. Gen. Appl. Microbiol. 26: 133–158.

Lopandic, K., Prillinger, H., Molnar, O. & Giménez-Jurado, G. (1996): Molecular characterization and genotypic identi-

fication of *Metschnikowia* species. Syst. Appl. Microbiol. 19: 393–402.

Maleszka, R. & Clark-Walker, G. D. (1990): Sequence of the gene for the cytoplasmic ribosomal RNA small subunit from *Kuyveromyces lactis*. Nucleic Acids Res. 18: 1889.

Mankin, A. S., Skryabin, K. G. & Rubtsov, P. M. (1986): Identification of ten additional nucleotides in the primary structure of yeast 18S rRNA. Gene 44: 143–145.

Melchers, W. J., Verweij, P. E., Hurk, P. van den, Belkum, A. van, De Pauw, B. E., Hoogkamp-Korstanje, J. A. & Meis, J. F. (1994): General primer-mediated PCR for detection of *Aspergillus* species. J. Clin. Microbiol. 32: 1710–1717.

Messner, R., Prillinger, H., Altmann, F., Lopandic, K., Wimmer, K., Molnar, O. & Weigang, F. (1994): Molecular characterization and application of Random Amplified Polymorphic DNA analysis of *Mrakia* and *Sterigmatomyces* species. Int. J. Syst. Bacteriol. 44: 694–703.

Messner, R., Schweigkofler, W., Ibl, M., Berg, G. & Prillinger, H. (1996): Molecular characterization of the plant pathogen *Verticillium dahliae* Kleb. using RAPD-PCR and sequencing of the 18SrRNA-gene. J. Phytopathol. 144: 347–354.

Mewes, H. W., Albermann, K., Bähr, M., Frishman, D., Gleissner, A., Hani, J., Heumann, K., Kleine, K., Maierl, A, Oliver S. G., Pfeiffer F. & Zollner A. (1997a): Overview of the yeast genome. Nature 387: 7–65.

Mewes, H. W., Albermann, K., Heumann, K., Liebl, S. & Pfeiffer, F. (1997b): MIPS: a database for protein sequences, homology data and yeast genome information. Nucleic Acids Res. 35: 28–30.

Moore, R. T. & Flinn, A. M. (1991): Ubiquinone systems in fungi I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. Trans. Mycol. Soc. Japan 26: 383–395.

Moore, T. D. E. & Edman, J. C. (1993): The a-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. Mol. Cell. Biol. 13: 1962–1970.

Morales, V. M., Jasalavich, C. A., Pelcher, L. E., Petrie, G. A. & Taylor, J. L. (1995): Phylogenetic relationship among several *Leptosphaeria* species based on their ribosomal DNA sequences. Mycol. Res. 99: 593–603.

Nagahama, T., Sato, H., Shimazu, M. & Sugiyama, J. (1995): Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA sequence. Mycologia 87: 203–209.

Nakase, T. & Suzuki, M. (1985): Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. I. Chemotaxonomic investigations. J. Gen. Appl. Microbiol. 31: 49–69.

Nishida, H., Ando, K., Ando, Y., Hirata, A., Sugiyama, J. (1995): *Mixia osmundae*: transfer from the Ascomycota to the Basidiomycota based on evidence from molecules and morphology. Can. J. Bot. 73 (Suppl. 1): S660–S666.

Nishida, H., Blanz, P. A. & Sugiyama, J. (1993): The higher fungus *Protomyces inouyei* has two group I introns in the 18S rRNA gene. J. Mol. Evol. 37: 25–28.

Nishida, H. & Sugiyama, J. (1993): Phylogenetic relationships among *Taphrina*, *Saitoella*, and other higher fungi. Mol. Biol. Evol. 10: 431–436.

Nishida, H. & Sugiyama, J. (1994): Archiascomycetes: detection of a major new lineage within the Ascomycota. Mycoscience 35: 361–366.

Noda, H. & Kodama, K. (1996): Phylogenetic position of yeastlike endosymbionts of anobiid beetles. Appl. Env. Microbiol. 62: 162–167.

O'Brien, R. W. & Ralph, B. J. (1966): The cell wall composition and taxonomy of some basidiomycetes and ascomycetes. Ann. Bot. 30: 831–843.

O'Donnell, K., Cigelnik, E., Weber, N. S. & Trappe, J. M. (1996): Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89: 48–65.

Ohkuma, M., Hwang, C. W., Masuda, Y., Nishida, H., Sugiyama, J., Ohta, A.& Takagi, M. (1993): Evolutionary position of n-alkane-assimilating yeast *Candida maltosa* shown by nucleotide sequence of small-subunit ribosomal RNA gene. Biosci. Biotechnol. Biochem. 57: 1793–1794.

Peer, Y. van de, Hendriks, L., Goris, A., Neefs, J. M., Vancanneyt, M., Kersters, K., Berny, J. F., Hennebert, G. & De Wachter, R. (1992): Evolution of basidiomycetous yeasts as deduced from small ribosomal subunit RNA sequences. Syst. Appl. Microbiol. 15: 250–258.

Percudani, R., Trevisi, A., Zambonelli, A. & Ottonello, S. (1999): Molecular phylogeny of truffles (Pezizales: Terfeziaceae, Tuberaceae) derived from nuclear rDNA sequence analysis. Mol. Phylogenet. Evol. 13: 169–180.

Prillinger, H., Deml, G., Dörfler, C., Laaser, G. & Lockau, W. (1991a): Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze: Hefe-Typen der Basidiomyceten. Teil II: *Microbotryum*-Typ. Bot. Acta 104: 5–17.

Prillinger, H., Dörfler, C., Laaser, G., Eckerlein, B. & Lehle, L. (1990a): Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze: Hefe-Typen der Basidiomyceten. Teil I: Schizosaccharomycetales, *Protomyces*-Typ. Z. Mykol. 56: 219–250.

Prillinger, H., Dörfler, C., Laaser, G. & Hauska, G. (1990b): Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze: Hefe-Typen der Basidiomyceten. Teil III: *Ustilago*-Typ. Z. Mykol. 56: 251–278.

Prillinger, H., Laaser, G., Dörfler, C. & Ziegler, K. (1991b): Ein Beitrag zur Systematik und Entwicklungsbiologie höherer Pilze: Hefe-Typen der Basidiomyceten. Teil IV: *Dacrymyces*-Typ, *Tremella*-Typ. Sydowia: 53: 170–218.

Prillinger, H., Lopandic, K., Schweigkofler, W., Deak, R., Aarts, H. J. M., Bauer, R. & Maraz, A. (2001, in press): Molecular phylogeny and systematics of the fungi with special reference to the Asco- and Basidiomycota. In: Breitenbach, M. & Crameri, R. (eds.) Chemical Immunology: Molecular Aspects of Fungal Allergy and Pathogenicity. S. Karger AG, Basel.

Prillinger, H., Oberwinkler, F., Umile, C., Tlachac, K., Bauer, R., Dörfler, C. & Taufratzhofer, E. (1993): Analysis of cell wall carbohydrates (neutral sugars) from ascomycetous and basidiomycetous yeasts with and without derivatization. J. Gen. Appl. Microbiol. 39: 1–34.

Prillinger, H., Schweigkofler, W., Breitenbach, M., Briza, P., Staudacher, E., Lopandic, K., Molnar, O., Weigang, F., Ibl, M. & Ellinger, A. (1997): Phytopathogenic filamentous

(*Ashbya, Eremothecium*) and dimorphic fungi (*Holleya*, *Nematospora*) with needle-shaped ascospores as new members within the Saccharomycetaceae. Yeast 13: 945–960.

Redhead, S. A. (1977): The genus *Neolecta* (Neolectaceae fam. nov., Lecanorales, Ascomycetes) in Canada. Can. J. Bot. 55: 301–306.

Saenz, G. S. & Taylor, J. W. (1999): Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. Mycol. Res. 103: 1049–1056.

Savile, D. B. O. (1955): A phylogeny of the basidiomycetes. Can. J. Bot. 33: 60–104.

Schweigkofler, W. (1998): Molekulare Identifizierung und Charakterisierung von endophytischen und latent pathogenen Pilzen aus Weinreben (*Vitis vinifera* L.) in Österreich und Südtirol. Ph.D. thesis, Univ. of Agriculture, Vienna.

Schweigkofler, W. & Prillinger, H. (1999): Molekulare Identifizierung und phylogenetische Analyse von endophytischen und latent pathogenen Pilzen der Weinrebe. Mitt. Klosterneuburg 49: 65–78.

Schweigkofler, W., Suzuki, M., Lopandic, K. & Prillinger, H. (2000): *Galactocandida mastotermitis* and *G. reticulitermites*: two new ascomycetous yeast species associated with termites. P. 186 in: Weber, H. C., Imhof, S. & Zeuske, D. (eds.) Proceedings of the Third International Congress on Symbiosis (TICS). Marburg, Germany.

Sjamsuridzal, W., Tajiri, Y., Nishida, H., Thuan, T., Kawasaki, H., Hirata, A., Yokota, A. & Sugiyama, J. (1997): Evolutionary relationships of members of the genera *Taphrina*, *Protomyces, Schizosaccharomyces*, and related taxa within the archiascomycetes: integrated analysis of genotypic and phenotypic characters. Mycoscience 38: 267–280.

Sogin, M. L., Miotto, K. & Miller, L. (1986): Primary structure of the *Neurospora crassa* small subunit ribosomal RNA coding region. Nucleic Acids Res. 14: 9540.

Sugiyama, J. & Nishida, H. (1995): The higher fungi: their evolutionary relationships and implications for fungal systematics. Pp. 177–195 in: Arei, R., Kato, M. & Doi, Y. (eds.) Biodiversity and Evolution. Natl Sci. Mus. Foundation, Tokyo.

Suh, S. O. & Sugiyama, J. (1993): Phylogeny among the basidiomycetous yeasts inferred from small subunit ribosomal DNA sequence. J. Gen. Microbiol. 139: 1595–1598.

Suzuki, M. & Nakase, T. (1986): Heterogeneity of ubiquinone systems in the genus *Sporothrix*. J. Gen. Appl. Microbiol. 32: 165–168.

Suzuki, M., Suh, S.-O., Sugita, T. & Nakase, T. (1999): A phylogenetic study on galactose-containing *Candida* species based on 18S ribosomal DNA sequences. J. Gen. Appl. Microbiol. 45: 229–238.

Swann, E. C. & Taylor, J. W. (1993): Higher taxa of Basidiomycetes: an 18S rRNA gene perspective. Mycologia 85: 923–936.

Swann, E. C. & Taylor, J. W. (1995): Phylogenetic diversity of yeast-producing basidiomycetes. Mycol. Res. 99: 1205–1210.

Tanabe, Y., O'Donnell, K., Saikawa, M. & Sugiyama, J. (2000): Molecular phylogeny of parasitic Zygomycota (Dimargaritales, Zoopagales) based on nuclear small subunit ribosomal DNA sequences. Mol. Phylogenet. Evol. 16: 253–262.

Teixeira A. Z., Iacomini, M. & Gorin, P. A. J. (1995): Chemotypes of mannose-containing polysaccharides of lichen mycobionts, a possible aid in classification and identification. Carbohydrate Res. 266: 309–314.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weigth matrix choice. Nucleic Acids Res. 22: 4673–4680.

Uijthof, J. M. J. & Hoog, G. S. de (1995): PCR-ribotyping of type isolates of currently accepted *Exophiala* and *Phaeococcomyces* species. Antonie van Leeuwenhoek 68: 35–42.

Walker, W. F. (1985): 5S ribosomal RNA sequences from Ascomycetes and evolutionary implications. Syst. Appl. Microbiol. 6: 48–53.

Walt, J. P. van der, Smith, M. T. & Yamada, Y. (1990): *Arxula* gen. nov. (Candidaceae), a new anamorphic, arthroconidial yeast genus. Antonie van Leeuwenhoek 57: 59–61.

Walt, J. P. van der & Yarrow, D. (1984): Methods for the isolation, maintenance, classification, and identification of yeasts. Pp. 45–104 in: Kreger-van Rij, N. J. W. (ed.) Theeasts, a Taxonomic Study. 3rd ed. Elsevier Sci. Publ., Amsterdam.

Wilmotte, A. M., Peer, Y. van de, Goris, A., Chapelle, S., De Baere, R., Nelissen, B., Neefs, J. M., Hennebert, G. L. & De Wachter, R. (1993): Evolutionary relationships among higher fungi inferred by small ribosomal subunit RNA sequence analysis. Syst. Appl. Microbiol. 16: 436–444.

Wong, O. C. & Clark-Walker, G. D. (1990): Sequence of the gene for the cytoplasmatic ribosomal RNA small subunit from *Candida (Torulopsis) glabrata*. Nucleic Acids Res. 18: 1888.

Yamada, Y. (1986). *Holleya* gen. nov., an ascosporogenous yeast genus for the Q9–equipped organism whose ascospores are needle-shaped with smooth surfaces on the anterior half and concentric ridges in their posterior half and without appendage. J. Gen. Appl. Microbiol. 32: 447–449.

Yamada, Y., Arimoto, M. & Kondo, K. (1973b): Coenzyme Q system in the classification of the ascosporogenous yeast genus *Schizosaccharomyces* and yeast-like genus *Endomyces*. J. Gen. Appl. Microbiol. 19: 353–358.

Yamada, Y., Arimoto, M. & Kondo, K. (1976a): Coenzyme Q system in the classification of apiculate yeasts in the genera *Nadsonia*, *Saccharomycodes*, *Hanseniaspora*, *Kloeckera* and *Wickerhamia*. J. Gen. Appl. Microbiol. 22: 293–299.

Yamada, Y., Arimoto, M. & Kondo, K. (1977): Coenzyme Q system in the classification of some ascosporogenous yeast genera in the families Saccharomycetaceae and Spermophthoraceae. Antonie van Leeuwenhoek 43: 65–71.

Yamada, Y., Banno, I., Arx, J. A. von & Walt, J. P. van der (1987): Taxonomic significance of the coenzyme Q system in yeasts and yeast-like fungi. Pp. 299–308 in: Hoog, G. S. de, Smith, M. T. & Wejman, A. C. M. (eds.) The Expanding Realm of Yeast-like Fungi. Elsevier, Amsterdam.

Yamada, Y., Kojiro, M., Banno, I. & Walt, J. P. van der (1992): An emendation of the genus *Debaryomyces* Lodder et Kruger-Van Rij and the proposals of two new combinations, *Debaryomyces carsonii* and *Debaryomyces etchellsii* (Saccharomycetaceac). J. Gen. Appl. Microbiol. 38:623–626.

Yamada, Y. & Kondo, K. (1972a): Taxonomic significance of coenzyme Q system in yeasts and yeast-like fungi (1). Pp. 363–373 in: Kocková-Kratachvílová, A. & Minarik, E. (eds.) Yeasts. Models in Science and Technics, Proc. 1st Spec. Int. Symp. on Yeasts. Slovak Acad. Scis, Bratislava.

Yamada, Y. & Kondo, K. (1972b): Taxonomic significance of the coenzyme Q system in yeasts and yeast-like fungi (2). Pp. 781–784 in: Terui, G. (ed.). Fermentation Technology Today, Proc. IVth Int. Ferment. Symp., Soc. Ferment. Technol. Osaka, Japan.

Yamada, Y. & Kondo, K. (1973): Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast genera *Sporobolomyces* and *Rhodosporidium*. J. Gen. Appl. Microbiol. 19: 59–77.

Yamada, Y., Nakase, T. & Walt, J. P. van der (1986): The coenzyme Q system in strains of species in the ascosporogenous yeast genera *Lipomyces* and *Waltomyces*. Trans. Mycol. Soc. Japan 27: 313–319.

Yamada, Y., Nakazawa, E. & Kondo, K. (1982): The coenzyme Q system in strains of *Trichosporon* species and related organisms. J. Gen. Appl. Microbiol. 28: 355–358.

Yamada, Y., Nojiri, M., Matsuyama, M. & Kondo, K. (1976b): Coenzyme Q system in the classification of the ascosporogenous yeast genera *Debaryomyces*, *Saccharomyces*, *Kluyveromyces,* and *Endomycopsis*. J. Gen. Appl. Microbiol. 22: 325–337.

Yamada, Y., Ohishi, T. & Kondo, K. (1983): The coenzyme Q system in strains of some yeasts and yeast-like fungi. J. Gen. Appl. Microbiol. 29: 51–57.

Yamada, Y., Okada, T., Ueshima, O. & Kondo, K. (1973a): Coenzym Q system in the classification of the ascosporogenous yeast genera *Hansenula* and *Pichia*. J. Gen. Appl. Microbiol. 19: 189–208.

Yamada, Y., Sasaki, J., Hou, C. F. Tahara, Y., Miranda, M. & Phaff, H. J. (1981): A new natural quinone, ubiquinone-5, isolated from an ascosporogenous yeast species *Nematospora coryli*. Agric. Biol. Chem. 45: 2671–2673.

Yamada, Y. & Smith, M. T. (1985): The coenzyme Q system in strains of species in the genera *Stephanoascus* and *Sporopachyderma* (Saccharomycetaceae). Trans. Mycol. Soc. Japan 26: 247–251.

Yamada, Y., Sugihara, K., Eijk G. W. van, Roeijmans, H. J. & Hoog, G. S. de (1989): Coenzyme Q systems in ascomycetous black yeasts. Antonie van Leeuwenhoek 56: 349–356.

Yamada, Y., Takinami-Nakamura, H., Tahara, Y. & Smith, M. T. (1980): The coenzyme Q system in the classification of the ascosporogenous yeast genus *Dekkera* and the asporogenous yeast genus *Brettanomyces*. Antonie van Leeuwenhoek 46: 595–599.