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Full Paper

Ribosomal DNA sequencing and reinstatement of the genus *Arthroascus* von Arx

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Sequence analysis of the D1/D2 domain of 26S rDNA was conducted upon seven *Arthroascus* strains from different geographic localities. The European and Asian species *Arthroascus schoenii* was documented from the North-American continent and from the Island of Hawaii. We discuss the heterogeneity of the genus *Saccharomycopsis* sensu Kurtzman and Robnett 1995. On the basis of molecular and genetic data the genus *Arthroascus* von Arx is reinstated.

Key Words——*Arthroascus schoenii*; D1/D2 domain; *Saccharomycopsis*; 26S rDNA sequence

Introduction

Many currently defined yeast genera are known to be heterogeneous and may contain aggregates of genetically closely related species. This may lead to dispute on generic delimitations. A good example of such a debated issue is the classification of the genera *Saccharomycopsis* Schiönning and *Arthroascus* von Arx. The type species of *Arthroascus* was originally described as *Endomyces javanensis* (Klöcker, 1909). The yeast was later assigned to different genera and renamed *Schwanniomyces javanensis* (Klöcker) Zender 1925, *Endomycopsis javanensis* (Klöcker) Dekker 1932 and *Schizosaccharomyces javanensis* (Klöcker) Streiblová 1967. The genus *Arthroascus* was proposed by von Arx (1972) to accommodate the single species *A. javanensis* (Klöcker) von Arx. A second species added was the long forgotten yeast *Endomyces schoenii* described by Nadson and Krassilnikov (1932). It was reclassified as *Arthroascus schoenii* (Nadson and Krassilnikov) Babjeva et al. on the basis of hybridization with *A. javanensis* and remained separate from that species because of the sterility of the hybrids (Bab'eva et al., 1986; Naumov et al., 1985). nDNA-DNA reassociation data confirmed this classification (Smith et al., 1990). Its close relatedness to *A. javanensis* was already noted in the first description of *A. schoenii* by Nadson and Krassilnikov (1932). It is worthy to note, that in the description of the genus *Arthroascus*, *A. schoenii* strain CBS 2556 (from H. J. Phaff) was used, along with the type culture of *A. javanensis* CBS 2555 (von Arx, 1972). A third species assigned to *Arthroascus*, *A. fermentans* Lee et al. from Taiwan, was identified as a separate taxon on the basis of the DNA-DNA reassociation data (Lee et al., 1994). Genetic hybridization analysis confirmed its generic placement and its status as a biological species (Naumov et al., 1999).

Kurtzman and Robnett (1995), using sequence data of the D1/D2 domain of large subunit (26S) rDNA, considered *Arthroascus* to be congeneric with the genus *Saccharomycopsis*. This classification was adopted by Kurtzman and Robnett (1998) and Kurtzman and Smith (1998). In the present paper, the relatedness among the species assigned to the *Saccharomycopsis*

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clade is reconsidered based on literature and our data.

Materials and Methods

Yeast strains. The list of *Arthroascus* strains and their origins are given in Table 1. More detailed information about the strains is available at http://www.cbs.knaw.nl and http://www.phaffcollection.org. UCD and UWO strains of *A. schoenii* were kindly supplied by H. J. Phaff and M.-A. Lachance.

Sequencing. DNA extraction was done as described previously (Naumov et al., 1997). The divergent D1/D2 domain at the 5′ end of the 26S rDNA gene was amplified with the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAG) and NL-4 (5′-GGTCCGTGTTTCAAGACGG) (O'Donnell, 1993). PCR amplification was performed using an MJ Research PTC-100 Programmable Thermal Controller for 36 cycles with denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 2 min. The size of the PCR product in all six strains studied was approximately 600 base pairs. The amplified DNA was purified using GFX-columns (Amersham Pharmacia Biotech., Inc., NJ, USA) according to the manufacturer's instructions. Direct sequencing of both strands of the D1/D2 region was performed using capillary electrophoresis on the ABI Prism 3700 automated DNA sequencer. The nucleotide sequences determined for seven *Arthroascus* strains have been deposited with GenBank (Table 1). Existing sequences for the type cultures of the *Arthroascus* species and other members of the *Saccharomycopsis* clade (Kurtzman and Robnett, 1995; Lachance et al., 2000) were retrieved from GenBank.

Alignment and phylogenetic analysis. Sequences were assembled using the SeqMan package (DNASTar, Inc., Madison, WI, USA). A preliminary automatic alignment was generated using BioNumerics version 1.50 (Applied Maths, Kortrijk, Belgium) and adjusted manually. The TREECON package (van de Peer and De Wachter, 1994) was used to generate a distance tree using the neighbor-joining algorithm with Kimura two-parameter correction. A total of 100 bootstrap replicates were used for analysis. The topology was verified using several algorithms (Parsimony, Ward's averaging, UPGMA).

Results and Discussion

We sequenced rDNA of seven *Arthroascus* strains

Table 1. *Arthroascus schoenii* strains studied.

Strain designation[a]		Source of isolation	Author	GenBank accession no. for D1/D2 sequences[b]
CBS	Other collections			
6423[c]	IFO 1579	Soybean-protein factory, Japan	T. Nakase	AY165964
6449	IFO 10681	Rotten tree trunk, Japan	K. Kodama	AY165963
7223 (T)[d]	VKM Y-1073	Oak bark, Kaluga, Russia	N. A. Krassilnikov	U40126
7425	CCRC 22504	*Panagrellus zymosiphilus* (nematode) on grape with sour rot, Italy	C. Schann	AY165962
9155	UCD 71-182	Ground dripping of flux of *Myoporum sandwicense*, Hawaii	H. J. Phaff	AY165961
9156	UCD 72-139	Flux of *M. sandwicense*, Hawaii	H. J. Phaff	AY165960
9159	UWO 80-91	*Quercus rubra*, Ontario, Canada	M.-A. Lachance	AY165958
9164	UWO 91-247.1	*Drosophila*, tequila factory, Mexico	M.-A. Lachance	AY165959

[a] The acronyms for culture collections are: CBS=Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; IFO=Institute for Fermentation, Osaka, Japan; VKM=All-Russian Collection of Microorganisms, Moscow, Russia; CCRC=Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan; UCD=Herman J. Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, USA; UWO=Department of Plant Sciences University of Western Ontario, London, Canada.

[b] All sequences are obtained in this study, except U40126.

[c] Ex-type culture of *Pichia nonfermentans* Nakase (1971).

[d] T=ex-type culture.

isolated in different geographic localities: Europe, North America, Far East Asia and Hawaii (Table 1). Comparative D1/D2 sequence analysis showed that all strains belonged to *A. schoenii*: five strains (CBS 6423, CBS 6449, CBS 7425, CBS 9159 and CBS 9164) had identical sequences with the type culture of *A. schoenii* CBS 7223 and two Hawaiian isolates (CBS 9155 and CBS 9156) exhibited two base differences (=0.35% sequence deviation). The study documents that *A. schoenii* has a world-wide distribution. Earlier *A. schoenii* isolates were known only from Europe and Far East Asia (Naumov et al., 1985; Smith et al., 1990). Note that strains CBS 9155, CBS 9156, CBS 9159 and CBS 9164 were originally designated as *A. javanensis*.

The D1/D2 sequences of the seven *A. schoenii* strains under examination were aligned with the corresponding sequences for sixteen species currently included in the *Saccharomycopsis* clade. Thirteen of them had been assigned previously to *Arthroascus*, *Endomycopsella*, *Guilliermondella* or *Botryoascus* (Kurtzman and Robnett, 1995, 1998). Lachance et al. (2000) added three *Candida* spp. to the *Saccharomycopsis* clade, but their phylogram did not include two recently described related species, *Saccharomycopsis microspora* and *Candida lassenensis* (Kurtzman, 1999). In the present report the phylogenetic analysis was expanded to compare all 16 members of the *Saccharomycopsis* clade, including the ex-type cultures of *A. javanensis*, *A. fermentans* and *A. schoenii* (Fig. 1).

It is evident from the expanded phylogenetic tree (Fig. 1) that the *Saccharomycopsis* clade is not monophyletic. Only four groups are statistically well-supported (bootstrap values 100%): three *Arthroascus* species, *Saccharomycopsis microspora* with *S. synnaedendra*, *S. crataegensis* with *Candida amapae*, and *S. selenospora* with *Candida* sp. UWO 91-121.1. These four lineages showed little phylogenetic affinity to each other or to any other species examined. The results suggest that the *Saccharomycopsis* clade appears to be composed of a complex of separate genera, one of which is *Arthroascus*. Earlier, phylogenetic analysis of partial 18S (positions 1451–1618, 168 bases) and 26S (positions 1611–1835, 225 bases, and positions 493–622, 130 bases) rRNAs from all three *Arthroascus* and six *Saccharomycopsis* species indicated that the two taxa are not congeneric (Yamada et al., 1996, 1998). The authors also concluded that the genus *Arthroascus* von Arx should be accepted. Morphologically, the members of the *Saccharomycopsis* clade are extremely different, showing various ascospore shapes (Kurtzman and Smith, 1998). The species classified earlier in *Arthroascus* genus can be well distinguished from the remaining taxa in the clade by the perforation of hyphal septa: cross walls with a closure line, i.e. the remnants of plasmalemma after centripetal closure during development of the wall (Kreger-van Rij, 1984). A morphological peculiarity to form ascospores with a ledge within swollen hyphal cells indeed unites the genetically related species *A. javanensis*, *A. schoenii* and *A. fermentans*, which have the same system of mating types responsible for their crossing. The resulting hybrids are sterile suggesting genetical isolation of the three biological species mentioned above (Naumov et al., 1985, 1999). The nDNA-nDNA reassociation data are in perfect agreement with

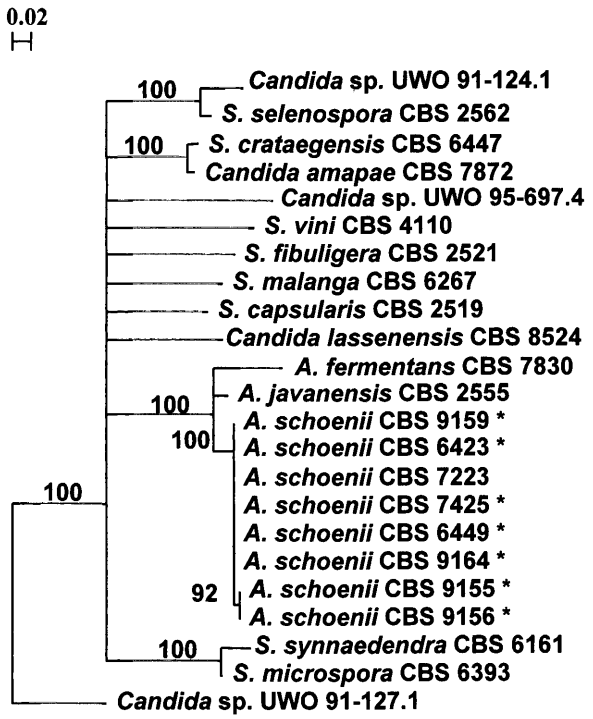

Fig. 1. Dendrogram showing phylogenetic relationship between the sixteen species within the *Saccharomycopsis* clade based on large subunit D1/D2 rDNA sequences.

The bootstrap values from 100 replications are shown as percentages (only values >90% are indicated). The scale bar shows the degree of sequence divergence (20 estimated base substitutions per 1,000 nucleotide positions). Existing sequences for the type cultures of 16 members of the *Saccharomyces* clade (Kurtzman, 1999; Lachance et al., 2000) were retrieved from GenBank. Sequences obtained in this study are marked by asterisks.

the genetic analysis. Different strains of the same species showed reassociation values of 85–100% and DNA similarity between the three species is 6.7–41% (Lee et al., 1994; Smith et al., 1990).

Thus, the genus *Arthroascus* contains hybridizing species and forms a well separated cluster within the so-called "*Saccharomycopsis* clade." Both genetic and molecular data argue in favor of reinstating of the genus *Arthroascus* von Arx (1972).

The other three statistically well-supported groups, as well as the remaining members of the so-called clade *Saccharomycopsis* sensu Kurtzman and Smith (1998), warrant some comments. Despite the heterogeneity of the clade, it does not seem expedient to separate monotypic genera. However, after positive studying of hybridization between the species *S. microspora* and *S. synnaedendra* it would be possible to describe a new genus on their basis. Development of taxonomy of the genus *Saccharomycopsis* would seem to depend on finding new species.

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