Arthroascus fermentans, a New Yeast Species Isolated from Soil in Taiwan

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***Arthroascus fermentans*, a new arthrosporous, fermenting, ascogenous yeast species, is described. The three strains of the species included in this study were isolated from soil samples obtained from different orchards in Taiwan. The species is homothallic, and the major ubiquinone isoprenolog is Q-8. DNA hybridization and DNA base composition data indicate that *A. fermentans* is a species that is distinct from previously described *Arthroascus* species. The type strain of *A. fermentans* is strain 80D2303, which has been deposited in the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, as strain CCRC 22530.**

The first species in the genus *Arthroascus* von Arx was originally described as *Endomyces javanensis* (5) in 1909. In 1931, this organism was placed in a new genus, *Endomycopsis* (9), and finally in 1972 it was assigned to another new genus, *Arthroascus* (12). In 1985, some strains of *Arthroascus javanensis* isolated from oak exudate in Russia were reclassified in a new species, *Arthroascus schoenii*, on the basis of differences in the nature of sporulation and in genetic characteristics from the type strain of *A. javanensis* (1).

During the isolation of yeasts from carambola and papaya orchard soils in Taiwan, we identified three strains of a previously undescribed yeast species belonging to the genus *Arthroascus*. These strains were characterized by the formation of budding cells, arthrospores, and spindle-shaped asci containing two to four flattened, spherical spores with thin ledges. The major ubiquinone was Q-8. Our isolates exhibited low levels of DNA relatedness to the previously described *Arthroascus* species. A new species, *Arthroascus fermentans*, is proposed for these yeasts.

MATERIALS AND METHODS

Yeast strains. The three strains which we studied, strains 80D2303^T (T = type strain), 80D2406, and 80D2412, were isolated in December 1991 from soil samples collected in a papaya orchard in I'lan, Taiwan, and in carambola orchards in Hualein, Taiwan. *A. schoenii* CCRC 21401 (= CBS 6423), CCRC 22503^T (= CBS 7223^T), and CCRC 22504 (= CBS 7425) and *A. javanensis* CCRC 21392^T (= CBS 2555^T) were used for taxonomic comparisons.

Examination of morphological, physiological, and biochemical characteristics. Morphological, physiological, and biochemical characteristics were determined by the methods described by van der Walt and Yarrow (11).

Morphological examination by scanning electron microscopy. Organisms were examined by scanning electron micro-

scopy by using a minor modification of the method described by Nakase et al. (7).

Cells grown in YPD broth (2% glucose, 2% peptone, 1% yeast extract) or agar blocks containing mycelia, liberated ascospores, and vegetative cells were prefixed in 2% glutaraldehyde and then fixed in a vapor consisting of 1% osmic acid in 0.1 M phosphate buffer (pH 7.2). The fixed specimens were gradually dehydrated by passing them at 15-min intervals through a graded series of acetone concentrations ranging from 30 to 100% and then were transferred to isoamyl acetate for more than 1 h. Finally, the samples were put in a model HCP-2 critical point drying apparatus (Hitachi) and dried.

The dried specimens were coated with gold at 1.2 kV and 5 mA in vacuo and were examined with a model 450 scanning electron microscope (Hitachi) operated at 15 or 20 kV.

Ubiquinone system. Cells were grown at 25°C in 100 ml of YPD liquid medium containing 1 g of yeast extract, 2 g of Bacto Peptone (Difco), and 2 g of glucose and were collected in the early stationary phase of growth. The cells were washed with distilled water, and ubiquinones were extracted and purified by the method of Collins et al. (3). Ubiquinone isoprenologs were identified by high-performance liquid chromatography (HPLC) by comparing the retention times of the compounds with the retention times of standard ubiquinones purchased from Sigma Chemical Co. HPLC was performed with a model 740 liquid chromatography apparatus (Waters) fitted with a Zorbax octyldecyl silane column (4.6 mm by 25 cm). The ubiquinones were eluted with a methanol-isopropanol (2:1, vol/vol) mobile phase at a flow rate of 1.0 ml/min and were detected at 275 nm (7).

DNA base composition. Cells were grown in YPD broth at 25°C and collected in the late logarithmic phase of growth. DNA was isolated and purified by the method of Kaneko and Banno (4). The purified DNA was hydrolyzed with nuclease P1 (Boehringer Mannheim Co.), and the resulting deoxyribonucleosides were digested with alkaline phosphatase (Sigma). The molar ratio of deoxyribonucleosides in the reaction mixture was determined by HPLC performed with a model 740 liquid chromatography apparatus (Waters) fitted with a Cosmosil C18 column (Nacalai Chemicals Co.). The deoxyribonucleosides were eluted with a mobile phase consisting of a 0.2

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FIG. 1. Morphology of *A. fermentans* 80D2303^T as determined by light microscopy (A and B) and scanning electron microscopy (C and D). The yeast strain was cultivated in 0.5% yeast extract–2% glucose broth for 3 days at 25°C. (A and B) Bars = 10 μ m. (C and D) Bars = 5 μ m.

FIG. 2. Morphology of *A. fermentans* 80D2303^T as determined by light microscopy (A and B) and scanning electron microscopy (C and D). The yeast strain was cultivated on YM agar at 25°C for 3 days. (A) Septa between cells and buds. (B) Elongate cells. (C) Tooth-like appendages around the mycelium. (D) Formation of arthrospores. (A, B, and D) Bars = 10 μ m. (C) Bar = 5 μ m.

FIG. 3. Dalmau plate culture of *A. fermentans* 80D2303^T grown on corn meal agar for 7 days at 25°C. (A) True mycelium. (B) Pseudomycelium. (C) Spindle-shaped asci. (D and E) Pseudomycelium containing spores. (A) Bar = 10 μ m. (B through E) Bars = 40 μ m.

M ammonium dihydrogen phosphate solution supplemented with 0.05 volume of acetonitrile. A standard deoxyribonucleoside mixture was prepared by digesting a standard deoxyribonucleotide DNA-GC kit (Yamasa Shoyu Co.) mixture with alkaline phosphatase (10).

DNA relatedness. DNA relatedness values were determined by the photobiotin-labeling microplate method described by Kaneko and Banno (4).

RESULTS

Latin diagnosis of *Arthroascus fermentans* sp. nov. In medio liquido post dies 3 cellulae singulae vel binae, cellulae ovoidae vel apiculatae (3.2–7.2 by 4.8–14.4 μ m). Post 1 mensem sedimentum formatur.

Cultura in agaro malti post 1 mensem (25°C), crenea vel brunneola, butyrosa, nitida, plana, nugosa, margine pilosa.

In agaro farinae *Zea mays* post dies 10 mycelium et pseudomycelium formantur.

Species homothallica. Asci conjugati vel inconjugati, habentes 2–4 sporae oblato-ellipsoidales aut lentiformis habens anulum tenuis in medio.

Asci deliquescent.

Fermentatio glucosi.

Glucosum, trehalosum (lente), glycerolum (lente), ethanolum assimilantur, at non galactosum, L-sorbosum, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, saccharosum, maltosum, α -methyl-D-glucosidum, cellobiosum, salicinum, N-acetyl-D-glucosaminum, arbutinum, melibiosum, lactosum, raffinose, melezitosum, inulinum, amyllum solubile, erythritolum, ribitolum, xylitolum, L-arabinitolum, D-glucitolum,

D-mannitolum, galactitolum, inositolum, glucono- δ -lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-gluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum DL-lacticum, acidum succinicum, acidum citricum, methanolum, acetonum, 2-propanolum, hexadecanum, nec ethyl acetat.

Lysinum assimilatur at non ethylaminum, cadaverinum, natrium nitricum, nec natrium nitrosum.

Augmentum in 30°C at non in 37°C.

Ad crescentiam vitaminarum externarum necessariae sunt.

Materia amyloidea iodophila non formatur.

Ureum non hydrolysatur.

Diazonium caeruleum B: negativum.

Proportio guanini + cytosini in acido deoxyribonucleati: 36.5–37.5 mol% (per HPLC).

Ubiquinonum primum: Q-8.

Typus ex terra in T'lan, Taiwan, isolata est. In Culture Collection and Research Center (CCRC), Food Industry Research and Development Institute, Hsinchu, Taiwan, sub no. 80D2303 (= CCRC 22530) deposita est.

Description of *Arthroascus fermentans* sp. nov. *Arthroascus fermentans* (fer' men. tans. L. part. adj. *fermentans*, fermenting, referring to the ability of the organism to ferment glucose vigorously). After growth in 0.5% yeast extract–2% glucose broth for 3 days at 25°C the cells are ellipsoidal, long ovoidal, and lemon shaped to elongate (3.2 to 7.2 by 4.8 to 14.4 μ m) (Fig. 1A and B). Buds are formed on broad bases at the extremities of the cells (Fig. 1C and D). A sediment is formed after 1 month.

After growth on YM agar (Difco) at 25°C for 3 days septa develop between buds and parent cells (Fig. 2A); short true

FIG. 4. Sporulation of *A. fermentans* 80D2303^T as determined by light microscopy (A and B) and scanning electron microscopy (C and D). The yeast strain was cultivated on acetate agar for 3 days at 25°C. (A) Conjugation and ascus formation. (B and C) Agglutinated spores. (D) Spore morphology. (A and B) Bars = 10 µm. (C) Bar = 5 µm. (D) Bar = 1 µm.

hyphae or pseudomycelia form (Fig. 2B), which break up into arthrospores (Fig. 2D). All strains produce short lateral dendrites (Fig. 2C).

After growth on 5% malt extract agar for 1 month at 25°C streak cultures are creamy to brownish, butyrous, dull, flat, wrinkled, and fringed with pseudomycelia.

On Dalmau plate cultures on corn meal agar after 10 days at 25°C abundant pseudomycelia and true mycelia with phase-dark septa develop (Fig. 3A and B). Some elongate cells are transformed into pouches, which usually contain four spores; the pouches (asci) become highly inflated in the middle (Fig. 3C through E).

Sporulation (formation of ascospores) occurs on YM agar, potato-dextrose agar, acetate agar, and malt extract agar after incubation at 25°C for 3 days, as well as in Dalmau corn meal plate cultures after 7 days. Cells may be transformed directly into asci, or conjugation between two cells occurs and the zygote inflates in the middle and is then transformed into a spindle-shaped ascus (Fig. 3C through E and 4A). Usually each ascus contains four spores; less frequently an ascus contains two spores. Each spore is oblate ovoidal and has a narrow equatorial ledge and smooth surface (Fig. 4C and D). As soon as the asci mature, the spores are liberated and agglutinate (Fig. 4B and C). All strains are homothallic, as determined by the method of Wickerham and Burton (13).

Ferments D-glucose, but does not ferment α,α-trehalose, raffinose, D-galactose, melibiose, inulin, maltose, lactose, starch, α-methyl-D-glucoside, cellobiose, D-xylose, sucrose, and melezitose.

Assimilates the carbon in D-glucose and ethanol. Assimilation of the carbon in glycerol is positive or latent. Assimilation of the carbon in α,α-trehalose is latent. Does not assimilate the carbon in soluble starch, DL-lactate, galactose, D-xylose, eryth-

ritol, succinate, L-sorbose, L-arabinose, ribitol, citrate, sucrose, D-arabinose, galactitol, myo-inositol, maltose, D-ribose, L-arabinitol, hexadecane, cellobiose, L-rhamnose, xylitol, 2-ketogluconate, D-glucosamine, D-mannitol, 5-ketogluconate, lactose, N-acetylglucosamine, D-glucitol, D-galacturonate, melibiose, D-glucono-1,5-lactone, α-methyl-D-glucoside, acetone, raffinose, arbutin, salicin, 2-propanol, melezitose, methanol, D-gluconate, ethylacetate, inulin, and D-glucuronate.

Does not assimilate the following nitrogen sources: nitrate, nitrite, ethylamine, and cadaverine. Assimilation of L-lysine is positive or weak.

Growth occurs at 30°C but not at 37°C.

Growth does not occur in vitamin-free medium.

Growth does not occur on 50% (wt/wt) glucose-yeast extract agar.

No starch-like substance is produced.

Does not liquefy gelatin.

Acid production on chalk agar is weak.

Grows in the presence of 1 mg of cycloheximide per ml.

Does not grow in the presence of 5% NaCl.

The G+C content of the nuclear DNA is 36.5 to 37.5 mol%, as determined by HPLC (Table 1).

Urease negative.

Does not hydrolyze casein.

The diazonium blue B reaction is negative.

Lipase negative.

The major ubiquinone is Q-8 (Table 2).

The type strain of *A. fermentans*, strain 80D2303, was isolated from soil from a papaya orchard in I'lan, Taiwan; strains 80D2406 and 80D2412 were isolated from soil samples from carambola orchards in Hualein, Taiwan. These strains have been deposited in the Culture Collection and Research Center, Food Industry Research and Development Institute,

TABLE 1. DNA base compositions and levels of DNA relatedness between species belonging to the genus *Arthroascus*

Species	Strain	G+C content (mol%)	% DNA relatedness to:	
			<i>A. fermentans</i> 80D2303 ^T	<i>A. javanensis</i> CCRC 21392 ^T
<i>A. fermentans</i>	80D2303 ^T	37.5	100	32
	80D2406	37.5	94	31
	80D2412	36.5	100	30
<i>A. javanensis</i>	CCRC 21392 ^T	37.5	29	100
<i>A. schoenii</i>	CCRC 21401	43.5	25	41
	CCRC 22503 ^T	41.6	18	32
	CCRC 22504	42.3	20	36

Hsinchu, Taiwan, as strains CCRC 22530, CCRC 22531, and CCRC 22532, respectively.

DISCUSSION

We classified the three newly isolated strains in the genus *Arthroascus* because they have characteristics typical of the genus *Arthroascus*, such as spindle-shaped asci, lemon-shaped or elongate cells, oblate ovoidal ascospores with ledges (6), a Q-8 ubiquinone system (Table 2), and a limited carbon assimilation pattern (2). However, these organisms differ from the two previously described *Arthroascus* species, *A. javanensis* and *A. schoenii*, in sporulation characteristics and fermentation ability. *A. javanensis* produces asci that contain one or (less frequently) two spores that have warty surfaces. The spores of this species clearly have a subequatorial ring, which imparts a helmet-shaped appearance to the spores (1). *A. schoenii* produces asci that contain two to four (sometimes six to eight) helmet-shaped ascospores with smooth surfaces (1). In *A. fermentans*, two to four oblate ovoidal spores with thin equatorial ledges and smooth surfaces are formed in each ascus. The diameter of *A. fermentans* spores (1.8 to 2.2 μ m) is less than the diameter of *A. javanensis* spores (4 μ m).

A. fermentans assimilates few carbon compounds, like the other *Arthroascus* species, but it ferments glucose vigorously, while the other *Arthroascus* species do not ferment this compound (2).

Additional evidence that *A. fermentans* represents a new species comes from the findings that *A. fermentans* differs from

A. schoenii in nuclear DNA base composition and that *A. javanensis* and *A. schoenii* exhibit low levels of DNA relatedness to the type strain of *A. fermentans* (Table 1). All of the *A. fermentans* strains exhibited high levels of DNA relatedness to the type strain of *A. fermentans*. Our genome comparison (Table 1) also revealed that *A. schoenii* is a species that is distinct from *A. javanensis*, as described by Smith et al. (8), although the physiological characteristics of the two species are very similar (2, 8).

Although the three strains of *A. fermentans* were isolated from soils, as was the only known strain of *A. javanensis*, the true habitats of these two species could be plant species growing in the soils. On the other hand, *A. schoenii* appears to occur in exudates of *Quercus* species in Russia, as well as in California (1).

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TABLE 2. Ubiquinone systems of *Arthroascus* species

Species	Strain	% of the following ubiquinone isoprenologs:		
		Q-6	Q-7	Q-8
<i>A. fermentans</i>	80D2303 ^T	2.2	7.3	90.5
	80D2406	0.0	0.0	100.0
	80D2412	0.0	5.6	94.4
<i>A. javanensis</i>	CCRC 21392 ^T	0.0	0.0	100.0
<i>A. schoenii</i>	CCRC 21401	0.0	0.0	100.0
	CCRC 22503 ^T	0.0	1.6	98.4
	CCRC 22504	0.0	2.1	97.9