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Lachancea meyersii sp. nov., an ascosporogenous yeast from mangrove regions in the Bahama Islands

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Abstract: *Lachancea meyersii* sp. nov. (type strain NRRL Y-27269, CBS 8951, ML 3925) is described from 18 strains collected from mangrove habitats in the northern Bahamas Islands. This species is homothallic, forms spherical ascospores in asci that become deliquescent, and is delineated from other ascomycetous yeasts by sequence analysis of the D1/D2 domains of the large subunit ribosomal DNA. The species can be distinguished from other members of the genus *Lachancea* by the combined characteristics of lack of growth on galactose and by growth on maltose. This new species is named in honor of Professor Samuel P. Meyers in recognition of his pioneering research with marine fungi.

Taxonomic novelty: *Lachancea meyersii* Fell, Statzell-Tallman & Kurtzman sp. nov.
Key words: yeast, systematics, mangroves, new species.

INTRODUCTION

Yeasts and other fungi are prevalent in marine salt marsh and mangrove ecosystems where they play an important role in the detrital food web (Hyde 2002, Meyers *et al*. 1975). One of the constraints on yeast ecology has been the questionable identities of the species due to the ambiguous taxonomic results based on phenotypic characterizations. This problem has been overcome by development of phylogenetic molecular data bases (Kurtzman & Robnett 1998, Scorzetti *et al.* 2002). Research at the University of Miami (UM) is presently exploring yeast community dynamics in the Florida Everglades, which has a range of environmental conditions from fresh water marshes to marine mangroves. The Everglades populations are being compared with yeasts previously collected in the mangroves of the Bahamas. An unknown ascomycetous yeast was a member the Bahamian yeast community. We propose to name this species *Lachancea meyersii*.

MATERIALS AND METHODS

Collections were made during UM research cruises in the Bahamas: May and Oct. 1996 to the Little Bahama Bank and Abaco Islands and July 1999 to Andros Island. One collection site was at Mangrove Cay (78º36'75 N, 26º55'00 W), which is on the Little Bahama Bank approximately 17 km north of Grand Bahama Island. Mangrove Cay is an island ~1.5 × 0.75 km that is flooded at each tide with the majority of the water funneled through a single deep creek. There is a shallow bar at the mouth of the creek that prohibits entry by small boats at low tide. Six stations, spaced through the ~1 km length of the creek were sampled near high tide (rising, falling or slack) with collections each day 19–22 May 1998 and 16 Oct. 1996. In the Abaco Islands, samples were obtained 24 and 25 May 1996 at Manjack Cay, (26º49'50" N, 77º21'80" W), an island ~ 2.5 km in length with an extensive mangrove basin and drainage creek. Additional samples were obtained 15 Jul. 1999 in the vicinity of Andros Island at Cross Cay (25º08'52" N, 78º14'50 W), which is a small mangrove island (~1 × 0.5 km) off the western coast of the NW corner of Andros.

At each collection site, 100–200 mL of water was filtered through 0.45 µm filters, which were placed on sea water medium (glucose 2 %, yeast extract 0.5 %, peptone 1 %, chloramphenicol 0.02 %, 2 % agar at 25 ºC) for colony growth and yeast isolation. Phenotypic characterizations of the strains followed the procedures of Yarrow (1998) with a modification: assimilation tests were run with 1 mL of test media in 2 mL microfuge tubes under constant rotation on a roller drum. All strains were identified from sequence analysis and 3 strains (CBS 8951, CBS 9924, CBS 9958) were selected for complete examination for phenotypic characters.

Molecular sequencing of the D1/D2 and ITS rDNA employed methods presented by Fell *et al*. (2000). Sequences were analysed phylogenetically by maximum parsimony and neighbor-joining with the Kimura 2-parameter distance correction using the pro-

grams of PAUP v. 4.063a (Swofford 1998). Sequence data for *Lachancea meyersii* (ML 3925, NRRL Y-27269, CBS 8951) were submitted to GenBank: D1D2 = AY645656, ITS = AY645657. Kurtzman & Robnett (2003) provided the GenBank numbers for the other species included in the analysis (Fig. 1).

RESULTS

Yeasts were generally abundant in waters adjacent to and within mangrove swamps. In the Mangrove Cay creek, the numbers ranged from 20 to 29000 cells/L with a diversity of ascomycetous and basidiomycetous yeast species. Similar results were obtained in the Florida Everglades mangroves and marshes. Identities of the species in the Bahamas and Everglades are being analysed and this publication is the first of a series of descriptions of new species from these regions.

The proposed new species, which is a member of the genus *Lachancea* (Fig. 1), is separated from known ascomycetous yeasts on the basis of phylogenetic analysis of nucleotide sequences from the ITS region and the D1/D2 domains of 26S rDNA. Eighteen strains were sequenced in the D1-D2 region and found to be identical, three strains were analysed in the ITS region and were also identical to each other. D1-D2 relationships between members of the genus *Lachancea* were analysed by maximum parsimony (MP) and from neighbour-joining (NJ) analyses. The trees were found to be congruent. A further analysis compared ITS sequences; the results demonstrated that the MP and NJ analyses gave ITS trees that were congruent with D1/D2 trees. Due to the absence of differences, the ITS tree is not presented in this publication.

TAXONOMY

Lachancea meyersii Fell, Statzell-Tallman & Kurtzman, **sp. nov.** MycoBank MB500091. Figs 2, 3.

Etymology: Lachancea meyersii is named in honor of Professor Samuel P. Meyers, Louisiana State University for his pioneering research with marine yeasts and filamentous fungi.

Cellulae globosae, ellipsoideae ad elongatae, singulae vel binae. Pseudomycelium nullum. Ascosporae per transformationem ascorum formantur aut per conjugationem cellularum vel cellulae et gemmae. Asci continentes 1–3 ascosporas globosae. Glucosum, sucrosum et threhalosum (exigue) fermentantur. Glucosum, sucrosum, maltosum, trehalosum, raffinosum, melezitosum, mannitolum, glucitolum, D-glucosidum, D-gluconatum (lente), ethanolum (exigue) et glycerolum (exigue) assimilantur.

Typus: CBS 8951 ex aqua maris in proximitate mangrovarum. Mangrove Cay, Bahama Islands, May, 1996. (type metabolically inactive in CBS).

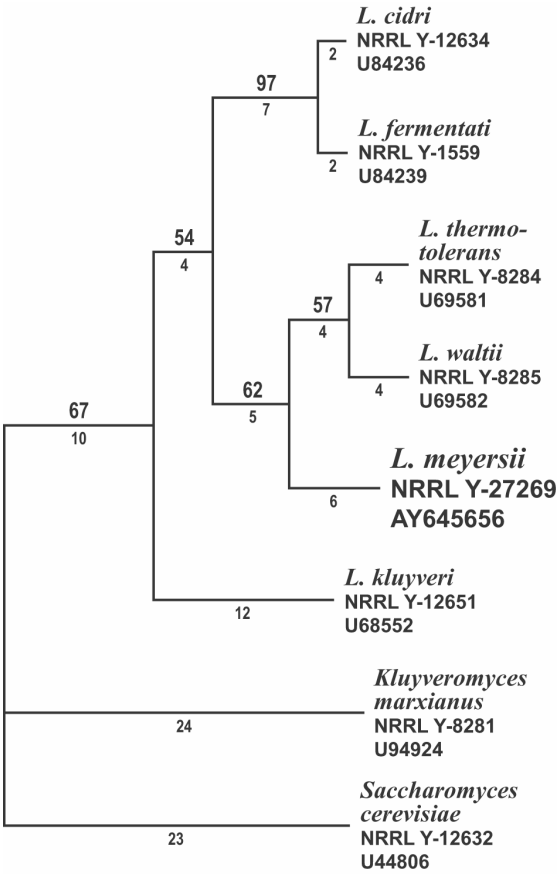


Fig. 1. Phylogenetic tree showing placement of *Lachancea meyersii* among species of the genus *Lachancea* with reference species *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* (outgroup species in the analysis) as represented by the single most parsimonious tree derived from maximum parsimony analysis of 26S rDNA domains D1 and D2. Branch lengths, proportional to nucleotide substitutions, are given below the branches and bootstrap values, based on 1000 replicates, are given above the branches. Frequencies under 50 % are not presented. Tree length = 107, consistency index = 0.832, retention index = 0.600, parsimony-informative characters = 28. GenBank accession numbers for previously described species were given by Kurtzman & Robnett (2003). All taxa are represented by type strains.

Growth on 5% malt extract agar: After 3 d at 25 ºC, the cells are often spherical (2.8–7 µm), or less frequently ellipsoidal (2.5–5.2 × 3–7 µm) to elongate, and occur singly or in pairs (Fig. 2). Budding is multilateral. Colonies are white to tannish-white, semi-glistening and butyrous.

Growth on 5% malt extract: After 3 d at 25 ºC, the cells are globose to subglobose or ovoid (2.7–5.4 × 3.4–6 µm) and may be single or in pairs.

Table 1. Fermentation and growth reactions for *Lachancea meyersii*.

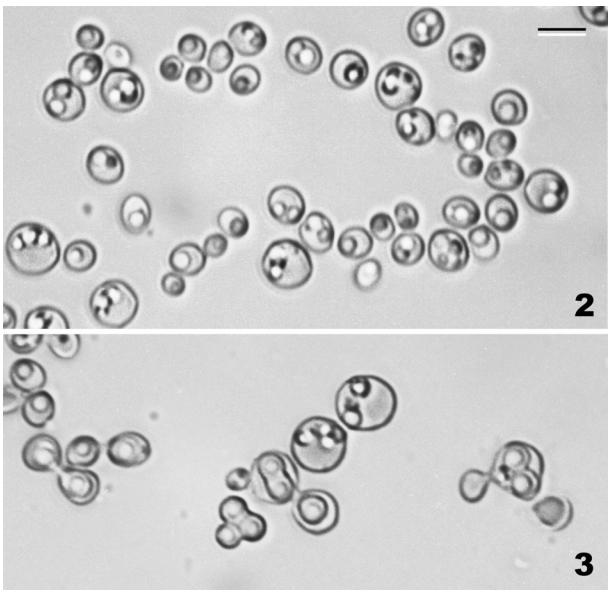
Fermentation[1]					
Glucose	+	Maltose	v	Trehalose	w
Galactose	–	Lactose	–		
Sucrose	+	Raffinose	v		
Assimilation[1]					
Glucose	+	L-Arabinose	–	D-Mannitol	+
Galactose	–	D-Arabinose	–	D-Glucitol	+
L-Sorbose	–	D-Ribose	–	∂-Methyl-	
Sucrose	+	L-Rhamnose	–	D-glucoside	+
Maltose	+	D-Glucosamine	–	Salicin	–
Cellobiose	–	*N*-Acetyl-D-		D-Gluconate	+s
Trehalose	+	glucosamine	–	DL-Lactate	–
Lactose	–	Methanol	–	Succinate	–
Melibiose	–	Ethanol	+D	Citrate	–
Raffinose	+	Glycerol	+D	Inositol	–
Melezitose	+	Erythritol	–	Hexadecane	–
Inulin	+D,–	Ribitol	V	Nitrate	–
Soluble starch	–	Galactitol	–	Vitamin-free	–
D-Xylose	–				
Additional assimilation tests and other growth characteristics					
2-Keto-D-gluconate	+s	DBB	–		
5-Keto-D-gluconate	–	Gelatin liquefaction	–		
Saccharate	+s	Growth at 37 °C:	–		
10 % NaCl / 5 % glucose	+	D-glucuronate:	–		
Starch formation	–	Cycloheximide 0.01 %	–		
Growth on 50 % (w/w) glucose yeast extract agar: v Cycloheximide 0.1 %	–				
Urease: –	–				
Creatinine: –	–				

[1] + = positive, – = negative, w = weak, v = strains variable, +s = positive, but slow, +D = positive, but delayed growth.

Dalmau plate culture on morphology agar: After 7 d at 25 ºC, neither pseudohyphae nor true hyphae are formed under the cover glass. Aerobic growth is white, semiglistening and butyrous in texture. Colonies are low convex with a depressed centre and with margins that are either smooth or finely and irregularly lobed.

Ascospore formation: Abundant ascosporulation occurred on 5 % malt extract agar after 6 d at 25 ºC. Some ascospores were also observed on YM agar, but not on yeast morphology and McClary's acetate agars. Asci may be unconjugated or show conjugation between independent cells or between a cell and its bud. Ascospores are spherical with 1–3 per ascus (Fig. 3). The presence of conjugation between cells and their buds suggests that the species is homothallic. Thirty-two single ascospores were isolated from NRRL Y-27269 by micromanipulation, but only three were viable. The resulting three colonies were ascosporogenous, and since they were derived from three-spored asci, the results further suggest that the species is homothallic.

Fermentation, assimilation and other growth characteristics: Results are given in Table 1.



Figs 2, 3. *Lachancea meyersii* NRRL Y-27269. 2. Budding cells, 5 % MEA, 3 d, 25 ºC. 3. Ascospore formation, 5 % MEA, 6 d, 25 ºC, showing conjugation between independent cells and between a cell and its bud. Asci are deliquescent at maturity. Bar = 5 µm for both figures.

Table 2. Salient characteristics in the genus *Lachancea*[1].

Assimilation	*L. meyersii*	*L. thermotolerans*	*L. waltii*	*L. cidri*	*L. fermentati*	*L. kluyveri*
D-Galactose	–	+	–	+	+	+
Maltose	+	+	–	+	+	+
Trehalose	+	+	–	+	+	+
Melezitose	+	+	–	–	+	v
∂-Methyl-D-glucoside	+	+	–	+	+	+
Succinate	–	v	–	+	+	–
DL-Lactate	–	-	–	+	+	+

[1]Data for *L. meyersii* from the present study, other species from Kurtzman & Fell (1998).

Origin of the strains examined: The strains were isolated from water samples at the surface to 1 m deep, near or among the mangrove prop roots of *Rhizophora mangle* trees in the Bahama Islands. Fourteen strains were isolated on consecutive days 19–22 May 1996 at 6 stations spaced through the mangrove creek on Mangrove Cay, Little Bahama Bank. Another isolation was made at the same location 16 Oct. 1996. Two strains were isolated May 1996 from a mangrove creek at Manjack Cay, Abacos, Bahamas and one strain was obtained from water adjacent to *Rhizophora mangle* roots of Cross Key, at the NW side of Andros Island, Bahamas July 1999. Salinity at all sites was ~37 %.

Type strain: CBS 8951 (ML 3925, NRRL Y-27269), isolated from seawater of Mangrove Cay, Bahamas, Little Bahama Bank (78º36'75N, 26º55'00W) in May 1996. Additional phenotypically characterized strains: ML 3937 (CBS 9958, NRRL Y-27411 collected 21 May 1996 at Mangrove Cay and ML 4115 (CBS 9924, NRRL Y-27762) collected 16 Oct 1996 at Mangrove Cay, Bahamas.

DISCUSSION

Lachancae meyersii shares many characteristics with other members of the genus in terms of cell shape, lack of hyphae and ascospore formation (Kurtzman 2003). All species currently accepted in the genus ferment glucose and sucrose, and assimilate raffinose, ethanol and mannitol, but not lactose, soluble starch, L- and D-arabinose, D-ribose, L-rhamnose, D-glucosamine, *N*-acetyl-D-glucosamine, methanol, erythritol, galactitol, citrate, inositol, hexadecane and nitrate. None of the species grow on vitamin-free media. *L. meyersii* is separated from other species of *Lachancea* by the combined characteristics of lack of growth on galactose and by growth on maltose (Table 2).

Lachancae meyersii is the first species in the genus collected from seawater habitats. The repeated isolation at various locations suggests that the species is a native inhabitant of Bahamian mangroves. All of the isolations of the other species of Lachancea have been from plants, plant products or plant-associated insects. The specific ecological niche of L. meyersii has not been determined. The habitat may be aquatic or possibly in conjunction with mangrove plants. This finding is not the first report of the genus in the Bahamas; L. thermotolerans was isolated from prickly pear cactus at Shroud Cay, Exumas (Lachance 1998).

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