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Candida amapae, a new amino acid-requiring yeast from the Amazonian fruit *Parahancornia amapa*

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SUMMARY

A new species of oligotrophic, methionine- or cysteine-requiring yeast was isolated from ripening fruits of the Amazonian *Parahancornia amapa* tree. This species resembles *Candida sorboxylosa* and species of *Issatchenkia* but is nonfermentative and forms abundant pseudomycelia. The new species was isolated only from *P. amapa* fruits, and not from other fruits or the drosophilid vectors of the yeast community of the deteriorating fruit. The taxon is regarded as indigenous to the amapa fruit or inoculated by vectors other than *Drosophila* before ripening. A description of the new species *Candida amapae* is given.

INTRODUCTION

The yeast community colonizing the fallen fruit of *Parahancornia amapa* (Huber) Ducke (Apocynaceae) is specific to this fruit in the Mocambo forest, Belém, Pará, Brazil. The amapa (*P. amapa*) is an endemic fruit tree of the upland and 'igapó' (seasonally flooded) forests of Amazonia. The fallen fruit is utilized as a feeding and oviposition site by a number of insects, including many drosophilids. Coexistence of the *Drosophila* Fállen species in the fallen fruit is at least partially mediated by successional colonization of the fruit by yeasts [7]. The most frequent isolates represented a *Candida sorbosa*-like complex, a *Candida sorboxylosa*-like complex, *Kloeckera apiculata* (Reess emend. Klöcker) Janke, *Kloeckera apis* Lavie ex Smith, Simione et Meyer, *Pichia kluyveri* Bedford ex Kudriavzev and a *Pichia membranaefaciens*-like species. *Candida krusei* (Castellani) Berkhout and a new species colonize the fruit during all phases of deterioration. The preceding two species were not isolated from the adult drosophilids that visit the fruit, and are probable vectors of the yeast community of the ripening fruit. The new species was not isolated from fruits of *Anacardium giganteum* (Anacardiaceae), *Clusia grandiflora* (Clusiaceae), *Helycostis* sp. (Moraceae) and *Platonia insignis* (Guttiferae) collected from the same forest site. Populations of the new species were around 1.0 to 4.0 $\times$ 10^6 CFU g^{-1} and represented about 10% of the total yeast isolates from the amapa fruit, and are regarded as indigenous to this substrate [7].

All of the 14 strains of the new species tested for physiological characteristics were very similar to *C. sorboxylosa* Nakase, and also to species of the genus *Issatchenkia* Kudriavzev. The most striking characteristic, which differentiates it from *C. sorboxylosa* and also from the *Issatchenkia* species, was an absolute requirement for the sulfur-containing amino acids methionine and cysteine.

MATERIALS AND METHODS

Fallen amapa fruit were collected during February–March of 1991 and 1992, in the Mocambo Forest Reserve, Belém, Pará, Brazil. Samples were collected aseptically in plastic bags, and taken to the laboratory at the Museu Paraense Emílio Goeldi within 3 h after collection [7]. Individual fruits were homogenized in a blender with 10% (w/v) of sterile physiological saline, and 0.5 g of each fruit was homogenized in a hand-held Teflon tissue homogenizer. Isolation of yeasts was done by the inoculation of decimal dilutions of fruit samples on acidified yeast extract-malt extract agar (YM agar: 0.3% yeast extract; 0.3% malt extract; 0.5% peptone; 2% glucose; and 2% agar). The medium was acidified by addition of 0.7% of 1 N HCl to a final pH of 3.5–4.0 and 100 mg L^{-1} of chloramphenicol was added. Plates were incubated at 25 $\pm$ 3 °C until colonies appeared. Counts of morphologically distinct colony types were then made, and a representative of each type was brought into pure culture by successive platings on YM agar for identification procedures [1,12]. Colonies of the new yeast could be easily recognized as white, dry and tough with a smooth center and cottony margins, and after three days

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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colonies became verrucose (wrinkled) with a fringed border of pseudomycelium. Cultures were maintained on 2% glucose, 0.5% yeast extract, 1% malt extract, 0.2% NaH_2PO_3, and 2% agar (GYMP agar) grown at 25 ± 3 °C and stored under sterile mineral oil at 8 ± 4 °C.

Characterization was done by standard methods [12]. Growth tests of carbon compounds were done on solid medium in petri dishes with 25 cultures inoculated per plate with a multitiped inoculating device and readings were made after 7, 14 and 21 days of incubation at 25 ± 3 °C. Tests for growth on nitrogen compounds, vitamin- and amino acid-requirements were done in broth media. DNA was isolated and purified based on the method of Marmur [3] as modified by Meyer and Phaff [5]. DNA base composition, expressed as G + C content, was calculated from the T_m (midpoint of the thermal transition) using the method of Marmur and Doty [4] and a Gilford Response II uv-vis spectrophotometer equipped with a model 2527 thermoprogrammer (Ciba Corning Diagnostics Corp., Gilford Systems, Oberlin, OH, USA). G + C contents were calculated from the T_m values by using the formula [5]: G + C content = $(T_m - 69.3)/0.41$. *Candida parapsilosis* DNA (T_m = 85.9 °C) was used as the standard.

RESULTS

Latin diagnosis of Candida amapae *sp. nov.*

In extracto malti post dies 3 ad 25 °C, cellulae vegetativae globosae aut elongatae, veut longae. Cellulae binae aut catenatae. Fiunt pseudohyphae. Post unum mensem ad 25 °C, sedimentum et anulus formantur.

In cultura in agaro malti post dies 3, cellulae cylindricae aut elongatae, pseudohyphae copiosa. Cultura alba, pilosa, mollis in centro cum margine undulato, butyrosum. Post dies 10, cultura cremea mollis cum margo rugosa lobatae et fimbriata.

In agaro farina *Zea maïs* post dies 5, pseudomycelium copiosum. Post dies 10–20, fiunt hyphae verae.

Asci et sporos non formantur.

Fermentatio glucosum nulla.

Glucosum, L-sorbosum, D-xylosum, D-glycerolum et acidum lacticum assimilantur. D-glucosaminum, acidum succinicum et acidum citricum assimilantur variabile est. D-galactosum, D-ribosum, D-arabinosum, L-arabinosum, L-rhamnosum, saccharum, maltosum, trehalosum, a-methyl-glucosidum, cellobiosum, salicinum, melibiosum, lactosum, raffinosum, melezitosum, inulinum, amylum solubile, i-erythritolum, ribitolum, 2-keto-gluconatum, L-arabinitolum, D-glucitolum, D-mannitolum, galactitolum, myo-inositolum, ethanolum, methanolum, ethylacetas et gluconas-d-lactonum non assimilantur.

Kalium nitricum et natrium nitrosum non assimilantur, nec ethyl aminum, L-lysinum et cadaverinum.

Ad crescentiam vitaminae externae, et cysteinum L-methioninum additae necessaria sunt.

Amylosum non formantur.

Augmentum non fit in temperatura 37 °C.

Urea non hydrolisatur.

Habitatio in fructus species *Parahancornia amapa*.

Typus: IM-UFRJ 51493 (= CBS no. 7872; ATCC no. 96298) cultura in agaro malti, designat stirpem typican. Isolata a fructo *Parahancornia amapa*.

G + C acidi deoxyribonucleati, 38.8 mol.%.

Standard description of Candida amapae

Growth on malt extract: After three days at 25 °C, the cells were large, globose to elongate, occasionally long. Cells in pairs or long chains of pseudohyphae. After four weeks, a sediment and thick ring formed.

Growth on malt extract-yeast extract agar: After three days, the cells were pleiomorphic, cylindrical to elongate, and with a well-developed and not strongly branched pseudomycelium. The streak culture was dry, white, cottony, rough with a smooth center and irregular fringed margins, and butyrous texture. After ten days, the colony was off-white, and smooth with rugose lobate margins fringed with pseudomycelium.

Dalmau plate cultures on corn meal agar: After five days, an abundant pseudomycelium developed, and after 10–20 days, rare true hyphae were observed.

Formation of ascospores: No ascospore formation or conjugation was observed in single or mixed cultures of 39 strains, on 2% or 5% malt extract agar, V-8 agar, corn meal agar and Gorodkowa agar, incubated at temperatures of 8, 18, 22, 28, and 35 °C.

Habitat: Ripe deteriorating fruits of *Parahancornia amapa* in the Amazon Rain Forest, Brazil.

Type strain: IM-UFRJ 51493 (AP 120 in Morais 1994 [7]) designated as type strain of *Candida amapae* is maintained in the Yeast Culture Collection of the Instituto de Microbiologia of the Universidade Federal do Rio de Janeiro, isolated from fallen fruit of *Parahancornia amapa*. It has been deposited in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, under no. 7872 and the American Type Culture Collection under no. 96298.

Results of growth tests and biochemical characterization are shown in Tables 1 and 2. The morphology of *C. amapae* including budding cells in glucose-yeast extract-peptone-water is shown in Fig. 1A and of filamentous growth with blastospores on corn meal agar is shown in Fig. 1B and C.

TABLE 2

Growth of *Candida amapae* on amino acids and precursors

Precursor added to basal medium[a]	Growth
None	−
SO_4^{2-}	−
SO_3^{2-}	−
$S_2O_3^{2-}$	−
Homoserine	+
Serine	+
Cysteine	+
Homocysteine	+
Methionine	+

[a]Yeast Nitrogen Base without amino acids (Difco Laboratories, Detroit, MI, USA) plus 0.5% glucose and precursor (10 mg L^{-1}).
Note: − = No growth after 7 days; + = Positive growth.

TABLE I

Growth and biochemical test results for *Candida amapae* sp. nov.

Fermentation					
Glucose	−				
Assimilation of carbon sources					
Glucose	+	D-xylose	+	α-methyl-D	
Galactose	−	D-arabinose	−	-glucoside	−
L-sorbose	+	L-arabinose	−	Glucano-	
Maltose	−	Ribose	−	δ-lactone	−
Sucrose	−	L-Rhamnose	−	DL-lactate	+
Cellobiose	−	Ethanol	−	Succinate	− (+)
Trehalose	−	Erythritol	−	Citrate	− (+)
Lactose	−	Methanol	−	Ethylacetate	−
Melibiose	−	Ribitol	−	Glucosamine	− (+)
Raffinose	−	Galactitol	−		
Soluble starch	−	Dulcitol	−		
Inulin	−	Mannitol	−		
Assimilation of nitrogen sources					
Nitrate	−	L-lysine	−		
Nitrite	−	Cadaverine	−		
Ethyl amine	−				
Growth without amino acid					
Methionine	−	Histidine	+	DL-tryptophane	+
Growth without vitamins	+				
Growth at 37 °C	−				
Osmotolerance: 10% NaCl	−				
Osmotolerance: 50% Glucose	−				
Biochemical tests					
Starch production	−				
DBB test	−				
G + C content	38.8 mol%				

Note: + = Positive growth; − = No growth; − (+) = Negative growth, with one strain positive.

DISCUSSION

This new anamorphic species is characterized by the requirement for methionine or other sulfur-containing amino acids, and it was placed in the heterogeneous genus *Candida* Berkhout. *Candida amapae* is DBB negative, and it resembles the *Issatchenkia* species. It can be distinguished from the known species of this genus by the lack of fermentative ability, and failure to form a pellicle on liquid media [2]. It also assimilates D-xylose and requires amino acids for growth. The phenotypic characteristics of the new species were also compared to other similar oligotrophic species of *Candida*, *C. krusei*, *C. sorbophila* (Nakase) Meyer et Yarrow, *C. sorbosa* Hedrick et Burke ex van Uden et Buckley and *C. sorboxylosa*. The lack of fermentation, assimilation of D-xylose and G + C content suggested similarity to *C. sorboxylosa* (G + C of 39.8 mol%). It can be distinguished from *C. sorboxylosa* by its requirement for methionine or cysteine, the abundance of pseudohyphae and presence of true hyphae, and absence of growth on succinic and citric acids [6]. Three strains of *C. amapae* presented variations in assimilation tests, one growing on D-glucosamine, one on succinic acid and the other on citric acid.

The requirement of amino acids is not a widespread characteristic among the yeasts [9]. It is similar in this respect to *Pichia amethionina* Starmer, Phaff, Miranda et Miller and *Pichia caribaea* Phaff, Starmer, Lachance, Aberdeen et Tredick-Kline, two cactophilic yeast species recovered from necrotic tissues of various cacti of the subtribes Stenocereinae and Pachycereinae in the North American Sonoran Desert and the Caribbean Islands [8,11]. The biosynthetic pathway for the sulfur-containing amino acids in yeasts requires the transport of SO_4^{2-} through the cell membrane, and includes SO_3^{2-}, $S_2O_3^{2-}$, homoserine, homocysteine, cysteine, and cystathionine as intermediary compounds. Serine is also a source of the carbon pool needed for the synthesis of methionine. The growth of *C. amapae* using different amino acids and precursors are shown in Table 2. The ability of *C. amapae* to grow with serine or homoserine but not with $S_2O_3^{2-}$ contrasts with the results of Starmer et al. [11], and suggests that its deficiency is somewhere in the sulfur-containing amino acid pathway and not the inability to convert hydrogen sulfite to hydrogen sulfide responsible for the methionine auxotrophy of *P. amethionina*. The differences in the production of pseudomycelia, the higher maximum temperature for growth of *P. amethionina*, and the higher G + C content of nuclear DNA

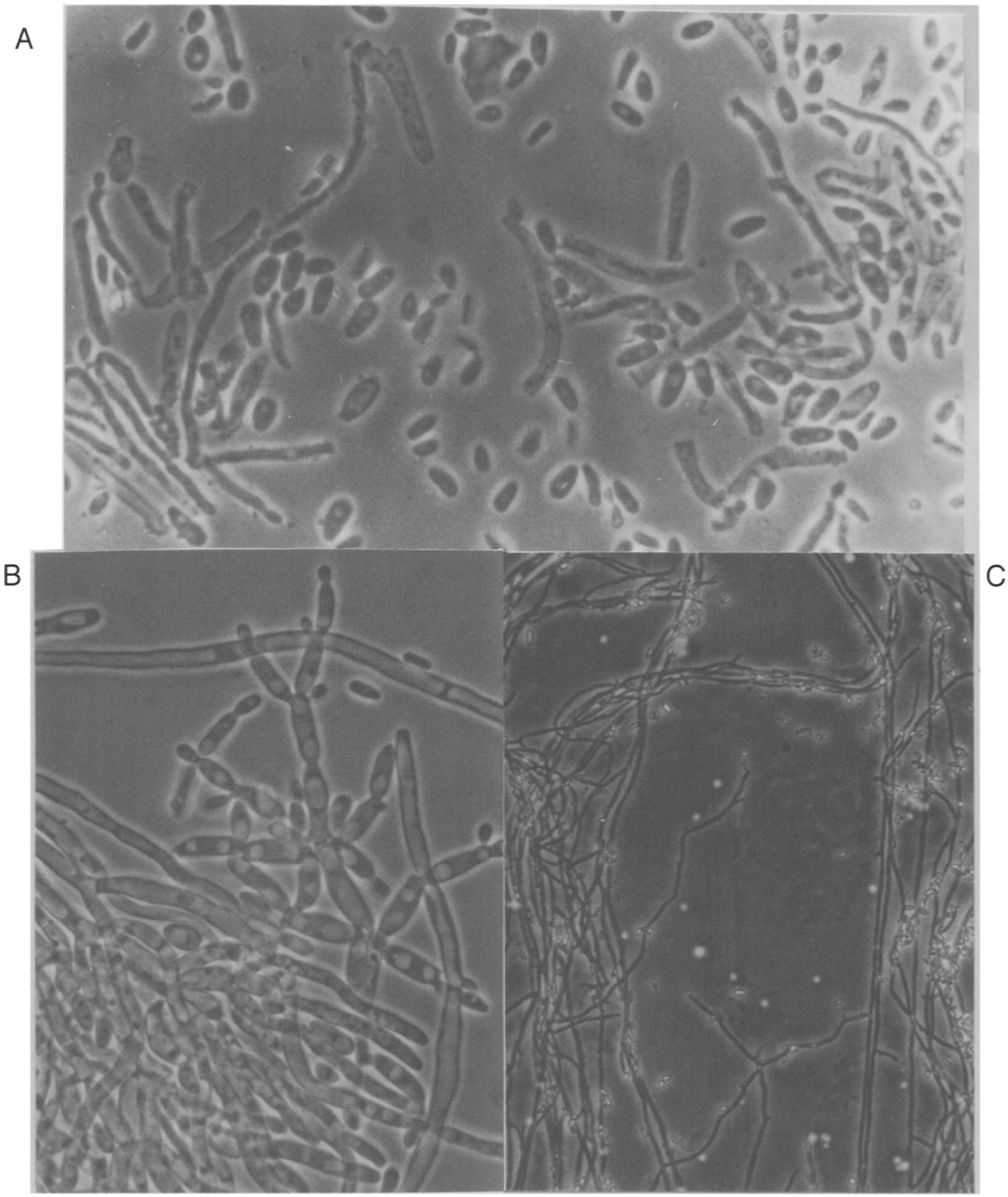

Fig. 1. Photomicrographs of *Candida amapae*. (A) Phase contrast 1200 × of a 3-day culture at 28 °C in 2% glucose -1% peptone -0.5% yeast extract water. (B) Phase contrast 1200 × of a 3-day culture grown at 28 °C on Corn Meal Agar. (C) Phase contrast 480 × of a 3-day culture grown at 28 °C on Corn Meal Agar.

of *C. amapae* (38.8 mol% when compared to 33.0–33.1 mol% of *P. amethionina* and 34.0–34.4 mol% of *P. caribaea*) makes it impossible for *C. amapae* to be an asporogenous variety of *P. amethionina* or *P. caribaea*. Also, their ecological specificities are sharply different, *C. amapae* being isolated from an Amazonian forest fruit and the two *Pichia* species being restricted to cactus habitats [7,8]. Four other species share this unusual property of methionine auxotrophy. Three are extensively filamentous species in the genera *Arthroascus* von Arx, *Guilliermondella* Nadson et Krassilnikov and *Saccharomycopsis* Schiönning [13] and one is an unidentified *Candida* species occurring in various plant exudates [10]. *Arthroascus javanensis* (Klöcker) von Arx has a G + C content of 29.5 mol% and presents variable growth on maltose, trehalose and soluble starch; and does not grow on L-sorbose, D-glycerol and DL-lactate. *Guilliermondella selenospora* Nadson et Krassilnikov has a G + C content of 30.2 mol%, fermentative ability and a wider physiological profile than *C. amapae*. It can grow on galactose, D-xylose, L-arabinose, ribose and ribitol as sole carbon sources. *Saccharomycopsis fibuligera* Klöcker has G + C content of 37.5 mol%, close to that of *C. amapae*, but it assimilates sucrose, maltose, cellobiose, raffinose, soluble starch, ribitol and mannitol, presents variable growth on trehalose, erythritol and inositol, ferments glucose, and grows at 37 °C.

The amapa fruit is a substrate rich in simple sugars, and the yeast community isolated from it presented a restricted physiological profile. These yeasts usually assimilated sorbose, D-xylose and organic acids. The aging of the fallen fruit and the successional growth of yeast colonizers probably caused a fall in the availability of nutrients to the yeasts. This affected some yeast species with restricted physiological abilities, but not *C. amapae*. It was able to colonize fruits of different ages, maintaining stable population numbers. This species seems to be adapted to the fruit environment, and is regarded as indigenous of the amapa fruit.

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