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Candida azymoides sp. n., a yeast species from tropical fruits and larva (Ascomycota) of *Anastrepha mucronota* (Diptera: Tephritidae)

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Abstract
Four strains of the new species *Candida azymoides* were isolated from larvae of *Anastrepha mucronota* (Diptera: Tephritidae) collected from ripe fruits of *Peritassa campestris* ("bacupari", Hippocrateaceae) in the state of Tocantins and from ripe fruits of *Eugenia uniflora* ("pitanga", Myrtaceae) collected in the state of Sergipe, Brazil. *Candida azymoides* is the sister species to *C. azyma* in the *Wickerhamiella* clade, in the Saccharomycetes. The type strain is *Candida azymoides* UFMG-R287 (CBS 10508).

Keywords: *Candida azymoides*, new yeast species, tropical fruits, *Anastrepha mucronota.*

Introduction

Decaying fruits are an important microhabitat for several yeast species (Phaff & Starmer, 1987; Morais et al., 1995; 2006; Ruivo et al., 2004). These ephemeral substrates are among the most important sites of oviposition and sources of nutrition for larval and adult stages of insects, which vector the yeasts to new substrates (Ganter, 2006; Morais et al., 2006). During a survey of yeasts associated with two Brazilian tropical fruits, *Peritassa campestris* (“bacupari”, Hippocrateaceae**)** and *Eugenia uniflora* (“pitanga”, Myrtaceae), and with associated insects, four strains of a new yeast species were isolated directly from fruits or from fly larvae found in the fruits. Analysis of the sequences of the internal transcribed spacer (ITS) and D1/D2 regions of the large subunit ribosomal DNA showed that these strains represent a distinct species that is a close relative of *Candida azyma*, in the *Wickerhamiella* clade. In this paper, we describe this new species as *Candida azymoides.*

Methods

The strains considered in this study are listed in Tab. 1. The samples of ripe fruits of *E. uniflora* were collected from small farms near the city of Aracajú in the state of Sergipe, as described by Trindade et al. (2002). Larvae of *Anastrepha mucronota* (Diptera: Tephritidae) were collected directly from decaying fruits of *P. campestris* in an “ipuca” ecosystem in the city of Lagoa da Confusão, state of Tocantins, in October 2005 (ipucas are unique forest fragments situated in the open fields of Cerrado vegetation and occur only in the Araguaia plains of state of Tocantins. These forest fragments are composed of a typical amazonic vegetation, and are flooded during about four to six months of the year). The larvae were collected with sterile forceps, surface sterilized by immersion in 70% ethanol for 1 min, and streaked out on plates containing yeast extract-malt extract agar (YM – 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, 2% agar, and 100 mg/L chloramphenicol). Some larvae were kept in the laboratory and reared to adult emergence for insect identification. Plates were incubated at room temperature (25 + 3ºC) for 3 to 8 days. Each different yeast morphotype was purified and maintained on YM slants or liquid nitrogen for later identification. The yeasts were characterized by standard methods (Yarrow, 1998). Identifications followed the keys of Kurtzman & Fell (1998).

The internal transcribed spacer (ITS) and D1/D2 regions of the large-subunit rDNA were amplified by PCR directly from whole cells as described previously (Marinoni & Lachance, 2004). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen, Mississauga, Ont., Canada) and sequenced using an ABI sequencer at the John P. Robarts Research Institute, London, Ontario, Canada. The sequence was edited with the program DNAMAN, version 4.1 (Lynnon BioSoft, Vaudreuil, QC, Canada). Existing sequences for other

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Table 1 - Origin of strains of *Candida azymoides*.

Strain numbers*	Substrate	Origin
UFMG-R287^T	Ripe fruit of *Eugenia uniflora*	Aracajú, Sergipe state
UFMG-R290	Ripe fruit of *E. uniflora*	Aracajú, Sergipe state
UFMG-05-T200.2	Larvae of *A. mucronota* in fruit of *P. campestris*	Ipuca ecosystem, Tocantins state
UFMG-05-T201.2	Larvae of *A. mucronota* in fruit of *P. campestris*	Ipuca ecosystem, Tocantins state

*Abbreviations: UFMG = Culture Collection of the Universidade Federal de Minas Gerais, Brazil; T = Type strain.

yeasts were retrieved from GenBank. The CLUSTAL W software (Thompson et al., 1994) incorporated in DNAMAN was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations. One strain from ripe fruit of *E. uniflora* and other from larva of *A. mucronota* were sequenced, and the ITS and D1/D2 sequences of the type strain has been deposited in GenBank under the accession number DQ985171.

Results and Discussion

Classification, ecology and species delineation

The sequence of the D1/D2 regions of the large subunit rDNA of *C. azymoides* differed by 7 (1.3%) substitutions from that of *C. azyma*, suggesting that they are closely related but separate yeast species (Fig. 1). The size of the D1/D2 sequences compared was 531 bp. Two strains of *C. azymoides* (UFMG-R287 and UFMG-05-T201.2) were sequenced, and they had identical sequences in ITS and D1/D2 domains of the large subunit rDNA. Ascomycetous yeasts that differ from one another by more than 1% substitutions in the D1/D2 domain of the large subunit rDNA usually represent distinct genetic species (Kurtzman & Robnett, 1998; Kurtzman & Fell, 2006). Strain ST-19, whose sequence was deposited in GenBank by S. Jindamorakot, S. Limtong, and T. Nakase differs by 7 substitutions from *C. azymoides*, but only 4 from *C. azyma*. Whereas it is not clear whether strain ST-19 should be assigned to *C. azyma*, we regard it as distinct from *C. azymoides*. The sequence of the ITS region of the type strain of *C. azymoides* differed by *ca.* 20 substitutions in ITS1 and four in ITS2 from that of *C. azyma* (unpublished results). These results show that *C. azymoides* is a sister species to *C. azyma* in the *Wickerhamiella* clade.

C. azymoides was originally isolated from ripe fruits of *E. uniflora* in the state of Sergipe, northeastern of Brazil, but was at that time identified physiologically as *Candida* sp. (Trindade et al., 2002). Additional isolates were later recovered from larvae of *A. mucronota* found in bacupari fruits *(P. campestris)*, suggesting that the yeast is also associated with this insect. *Anastrepha mucronata* is a frugivorous fly, and this is the first record of its larva in Brazilian fruits (Bomfim et al., 2004). The fly species is not known to be economically important, but other species in the genus are considered pests for many different kinds of fruits (Cruz-López et al., 2006). Although *A. mucronota* was collected from fruits of *P. campestris*, it probably attacks other fruits in the ipuca ecosystem. For this reason the true habitat of *C. azymoides* could be *A. mucronota* and the tropical fruits that it colonizes. The sister species of *C. azymoides*, *C.*

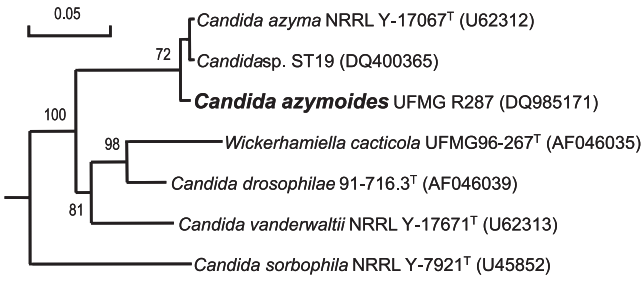

Figure 1 - Neighbour joining phylograms based on the D1/D2 divergent domains of the large subunit rDNA of *Candida azymoides* and their closest relatives. The percentage bootstrap values were obtained from 1000 iterations. The scale bar shows 5% sequence divergence. All strains shown are type strains.

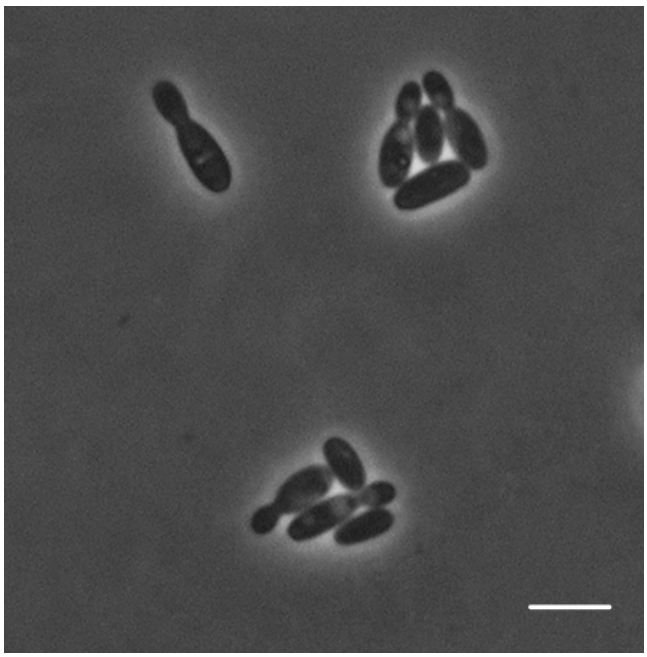

Figure 2 - Phase contrast micrographs of vegetative cells of *Candida azymoides* growing on yeast extract-malt extract (YM) agar at 22ºC after 3 days. Bar = 5 mm.

azyma, is an asexual yeast occurring in ephemeral flowers throughout the New World and Australian region (Lachance et al., 2001).

The four isolates of *C. azymoides* were examined after growth on the most common sporulation media (corn meal agar, dilute V8 agar, 5% malt extract agar, and Yeast Carbon Base agar supplemented with 0.01% ammonium sulphate) alone or mixed in pairs, but asci or signs of conjugation were not seen, indicating that the species occurs in nature in the asexual form. In terms of growth characteristics, *C. azymoides* differs from *C. azyma* only by the absence of growth on α-methyl-D-glucoside as sole carbon source. Other minor variations occur within either species but cannot be used for identification.

Latin diagnosis of Candida azymoides ***Rosa, Morais, Lachance & Trindade sp. n.***

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (2-3 x 2-4 mm*). Post unum mensem sedimentum formatur. Cultura in agaro malti post dies 14 (17ºC) parva, convexa, glabra et candida. In agaro farinae Zea mays post dies 14 mycelium nec pseudomycelium non formantur. Glucosum non fermentatur. Glucosum, sucrosum, galactosum, trehalosum, maltosum (lente), melezitosum, L-sorbosum, D-xylosum (lente), L-arabinosum (lente), ethanolum (lente), glycerolum, ribitolum, xylitolum (lente), mannitolum, glucitolum, acidum succinicum, acidum citricum, 2-keto-gluconatum assimilantur, at non inulinum, raffinosum, melibiosum, lactosum, α-methyl-D-glucosidum, amylum solubile, cellobiosum, salicinum, L-rhamnosum, D-arabinosum, D-ribosum, methanolum, erythritolum, galactitolum (aliquando exigue), meso-inositolum, acidum lacticum, acidum gluconicum, glucosaminum, N-acetylglucosaminum, xylitolum, acetonum, ethyl acetas, nec hexadecanum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum. Ad crescentiuam vitaminae externae necessariae sunt. Augmentum in 30ºC, at non 37ºC. Habitat fructos Eugenia uniflora et Peritassa campestris in Brazil. Typus UFMG-R287. In collectione zymotica* Centraalbureau voor Schimmelcultures, *Trajectum ad Rhenum, sub no. CBS 10508 typus stirps deposita est.*

Description of *Candida azymoides* Rosa, Morais, Lachance & Trindade sp. n.

In yeast extract (0.5%), glucose (2%) broth after 3d at 25ºC, the cells are ovoid to ellipsoidal (2-3 x 2-4 mm). Budding is multilateral. A sediment is formed after a month, but no pellicle was observed. On YM agar after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, neither pseudomycelia nor true mycelia are formed. Glucose is not fermented. Assimilation of carbon compounds: glucose, sucrose, galactose, trehalose, maltose, melezitose, sorbose, D-xylose (slow), L-arabinose (slow), ethanol (slow), glycerol, ribitol, xylitol, D-mannitol, D-glucitol, succinic acid, citric acid and 2-keto gluconate. No growth occurs on inulin, raffinose, melibiose, lactose, α-methyl-D-glucoside, soluble starch, cellobiose, salicin, L-rhamnose, D-arabinose, D-ribose, methanol, isopropanol, 2-propanol, erythritol, galactitol (sometimes weak), myo-inositol, lactic acid, gluconic acid, glucosamine, N-acetyl-glucosamine, acetone, ethyl acetate, and hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine, and negative for nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 30°C is positive; at 37°C is negative. Growth on YM agar with 5% sodium chloride is positive, and at 10% sodium chloride is variable. Growth in 50% glucose/yeast extract (0.5%) is negative or weak. Starch-like compounds are not produced. In 1000 mg cycloheximide mL^{-1} growth is positive. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is fruits in Brazil. The type strain accession number of *Candida azymoides* is UFMG-R287^T. It was isolated from a ripe fruit of *Eugenia uniflora* in the state of Sergipe, Brazil, and has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as strain CBS 10508^T.

The epithet *azymoides* (a.zy.mo'i.des) L. nom. fem. sing. adj. azymoides, refers to the similarity to *C. azyma.*

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References

Bomfim, D. A.; Uchôa-Fernandes, M. A. & Bragança, M. A. L. 2004. Espécies de moscas frugívoras (Diptera: Tephritoidea) no Estado do Tocantins. In: **Resumos do XX Congresso Brasileiro de Entomologia**. Gramado, RS. 2004. pp. 655.

Cruz-López, L.; Malo, E. A.; Toledo, J.; Virgen, A.; Del Mazo, A. & Rojas, J. C. 2006. A new potential attractant for *Anastrepha obliqua* from *Spondias mombin* fruits. **Journal of Chemical Ecology, 32:** 351-365.

Ganter, P. F. 2006. Yeast and invertebrate associations. In: Rosa, C. A. & Peter, G. (Ed.) **Biodiversity and Ecophysiology of Yeasts, The Yeast Handbook**. Heidelberg, Springer, pp. 303-370.

Kurtzman, C. P. & Fell, J. W. 1998. **The Yeasts, a Taxonomic Study**. Fourth Ed. Amsterdam, Elsevier, 1055 pp.

Kurtzman, C. P. & Fell, J. W. 2006. Yeast systematics and phylogeny – implications of molecular identification methods for studies in ecology. In: Rosa, C. A. & Peter, G. (Ed.) **Biodiversity and Ecophysiology of Yeasts, The Yeast Handbook**. Heidelberg, Springer, pp. 12-30.

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.

Lachance, M. A.; Starmer, W. T.; Rosa, C. A.; Bowles, J. M.; Barker, J. S. & Janzen, D. H. 2001. Biogeography of the yeasts of ephemeral flowers and their insects. **FEMS Yeast Research, 1:** 1-8.

Marinoni, G. & Lachance, M. A. 2004. Speciation in the large-spored *Metschnikowia* clade and establishment of a new species, *Metschnikowia borealis* comb. nov. **FEMS Yeast Research, 4:** 587-596.

Morais, P. B.; Martins, M. B.; Hagler, A. N.; Mendonça-Hagler, L. C. & Klaczko, L. B. 1995. Yeast succession in the Amazon fruit *Parahancornia amapa* as resource partitioning among *Drosophila* spp. **Applied and Environmental Microbiology, 61:** 4251-4257.

Morais, P. B.; Pagnocca, F. C. & Rosa, C. A. 2006. Yeast communities in tropical rain forests in Brazil and other South American ecosystems. *In*: Rosa, C. A. & Peter, G. (Ed.) **Biodiversity and Ecophysiology of Yeasts. The Yeast Handbook**. Heidelberg, Springer, pp.461-484.

Phaff, H. J. & Starmer, W. T. 1987. Yeasts associated with plants, insects and soil. *In*: Rose, A. H. & Harrison, J. S. (Ed.). **The Yeasts, biology of yeasts**. London, Academic Press, pp. 123-180.

Ruivo, C. C. C.; Lachance, M. A.; Bacci Jr., M.; Carreiro, S. C.; Rosa, C. A. & Pagnocca, F. C. 2004. *Candida leandrae* sp. nov., an asexual ascomycetous yeast species isolated from tropical plants. **International Journal of Systematic and Evolutionary Microbiology, 54:** 2405-2408.

Thompson, J. D.; Higgins, D. G. & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment throught sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Research, 22:** 4673-4680.

Trindade, R. C.; Resende, M. A.; Silva, C. M. & Rosa, C. A. 2002. Yeasts associated with fresh and frozen pulps of Brazilian tropical fruits. **Systematic and Applied Microbiology, 25:** 294-300.

Trindade, R. C.; Resende, M. A.; Pimenta, R. S.; Lachance, M. A. & Rosa, C. A. 2004. *Candida sergipensis*, a new asexual yeast species isolated from frozen pulps of tropical fruits. **Antonie van Leeuwenhoek, 86:** 27-32.

Yarrow, D. 1998. Methods for the isolation and identification of yeasts. In: Kurtzman, C. P. & Fell, J. W. **The Yeasts, a Taxonomic Study.** Amsterdam, Elsevier pp. 77-100.