

# *Spathaspora brasiliensis* sp. nov., *Spathaspora suhii* sp. nov., *Spathaspora roraimanensis* sp. nov. and *Spathaspora xylofermentans* sp. nov., four novel D-xylose-fermenting yeast species from Brazilian Amazonian forest

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**Abstract** Four new D-xylose fermenting yeast species of the clade *Spathaspora* were recovered from rotting-wood samples in a region of Amazonian forest, Northern Brazil. Three species produced unconjugated asci with a single elongated ascospore with curved ends. These species are described as *Spathaspora brasiliensis*, *Spathaspora suhii* and *Spathaspora roraimanensis*. Two isolates of an asexually

reproducing species belonging to the *Spathaspora* clade were also obtained and they are described as *Spathaspora xylofermentans*. All these species are able to ferment D-xylose during aerobic batch growth in rich YP (1 % yeast extract, 2 % peptone and 2 % D-xylose) medium, albeit with differing efficiencies. The type strains are *Spathaspora brasiliensis* sp. nov. UFMG-HMD19.3 (=CBMAI 1425=CBS 12679), *Spathaspora suhii* sp. nov. UFMG-XMD16.2 (=CBMAI 1426=CBS 12680), *Spathaspora roraimanensis* sp. nov. UFMG-XMD23.2 (CBMAI 1427=CBS 12681) and *Spathaspora xylofermentans* sp. nov. UFMG-HMD23.3 (=CBMAI 1428=CBS 12682).

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## Introduction

Bioethanol production from lignocellulosic material uses substrates, such as agricultural residues and wood waste, that do not compete with food supplies and therefore presents an economic and environmentally sustainable opportunity (Hahn-Hägerdal et al. 2006; Solomon 2010; Cheng and Timilsina 2011). In lignocellulosic feedstocks the second most abundant sugar after glucose is the pentose xylose, and although several yeast species have been shown to be able to

ferment this sugar (including recombinant *Saccharomyces cerevisiae* strains), the efficiency and rates of xylose fermentation are low, challenging the feasibility of industrial production of lignocellulosic bioethanol (Hahn-Hägerdal et al. 2007; Stambuk et al. 2008; Amorim et al. 2011). Therefore, there is still a need for new yeasts capable of efficient xylose fermentation for bioethanol production.

The yeast genus *Spathaspora* contains two sexually-reproducing species, *Spathaspora passalidarum* and *Spathaspora arborariae*, that produce elongate ascospores with curved ends (Nguyen et al. 2006; Cadete et al. 2009). These species are associated with rotting-wood substrates and the insects that occupy this ecological niche. Both species are known for their ability to ferment D-xylose producing ethanol and have some potential for the production of biofuels through fermentation of plant cell wall materials (da Cunha-Pereira et al. 2011; Nguyen et al. 2011). The asexual species *Candida insectamans*, *Candida jeffriesii*, *Candida lyxosophila*, *Candida materiae*, and *Candida subhashii* also belong to the *Spathaspora* clade and are also associated with wood substrates or insects (Barbosa et al. 2009; Lachance et al. 2011; Nguyen et al. 2011). Da Cunha-Pereira et al. (2011) reported on the possible use of *S. arborariae* alone or in co-culture with *S. cerevisiae* for ethanol production using rice hull hydrolysates. *S. arborariae* was able to metabolize the xylose and the glucose present in the hydrolysate with an ethanol conversion yield of 0.45 g g<sup>-1</sup>. *S. passalidarum* simultaneously coferments glucose, cellobiose, and xylose with an ethanol yield of 0.42 g g<sup>-1</sup>, and it has a specific ethanol production rate on xylose more than 3 times that of the corresponding rate on glucose (Long et al. 2012). Xylose-fermenting species of the *Spathaspora* clade could be used directly for ethanol production or might provide a source of genes, enzymes and/or sugar transporters to engineer industrial strains for the efficient production of bioethanol from renewable biomass (Hahn-Hägerdal et al. 2007; Wohlbach et al. 2011).

During an extensive study of yeasts associated with rotting wood in Brazilian ecosystems, we isolated six D-xylose-fermenting yeasts. Sequence analyses of the ITS region and D1/D2 domains of the large subunit rRNA gene showed that these isolates represented four new species belonging to the clade *Spathaspora*. Three species produced ascospores with curved ends and are here described as *Spathaspora brasiliensis* sp.

nov, *Spathaspora suhii* sp. nov. and *Spathaspora roraimanensis* sp. nov. Two isolates representing an asexual *Spathaspora* species closely related to *S. roraimanensis* are described here as *Spathaspora xylofermentans* sp. nov.

## Materials and methods

### Yeast isolation and identification

The yeasts were isolated from samples of rotting wood collected in two areas of Amazonian forest in the state of Roraima, Northern Brazil. The areas are the site of a long-term experiment maintained by Embrapa-Roraima, located in the municipalities of São João da Baliza (00°56'58"N and 59°54'41"W) and Mucajái (2°25'48"N and 60°55'11"W). The predominant vegetation is characterized as an Amazonia Forest biome. The climate is hot and humid, with an annual precipitation between 1,300 to 2,900 mm, and an average temperature from 25.6 to 27.6 °C. Forty decayed wood samples were collected, twenty from each area, in October 2009. The samples were stored in sterile plastic bags and transported under refrigeration to the laboratory over a period of no more than 24 h. One gram of each sample was placed separately in 125 mL Erlenmeyer flasks with 20 mL sterile D-xylose medium (yeast nitrogen base 0.67 %, D-xylose 0.5 %, chloramphenicol 0.02 %) or 20 mL sterile xylan medium (yeast nitrogen base 0.67 %, xylan 1 %, chloramphenicol 0.02 %, pH 5.0 ± 0.2), respectively. The flasks were incubated at 25 °C in an incubator shaker (New Brunswick) at 150 rpm for 3–10 days. When growth was detected, 0.5 mL of a culture was transferred to a tube containing 5 mL sterile D-xylose or xylan medium, and the tubes were incubated on an incubator shaker as described above. After growth detection, one loopful of each tube was streaked on D-xylose or xylan agar medium. The plates were incubated at 25 °C until yeast colonies developed (Cadete et al. 2009). The different yeast morphotypes were purified by repeated plating on YM agar (glucose 1 %, peptone 0.5 %, yeast extract 0.3 %, malt extract 0.3 %, agar 2 %) and maintained on GYMP broth (2 % glucose, 0.5 % yeast extract, 0.5 % malt extract, 0.2 % mono sodium phosphate) with 20 % glycerol at -80 °C or in liquid nitrogen for later identification. The yeasts were characterized by

standard methods (Kurtzman et al. 2011a). Preliminary identities were determined using the keys of Kurtzman et al. (2011b). Sporulation was investigated using 1 % glucose–0.01 % yeast extract (GY), Yeast Carbon Base plus 0.01 % ammonium sulfate (YCBAS), dilute (1:9 and 1:19) V8, Fowell's acetate and YM agars at 20 °C for up to four weeks (Cadete et al. 2009).

#### DNA sequencing and sequence analysis

The ITS1-5.8S-ITS2 region and the D1/D2 variable domains of the large-subunit rRNA gene were amplified by PCR directly from whole cells using primers ITS1 (5'-TAG GTG AAC CTG CGG AAG GAT CAT-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3'), as described previously (Marinoni and Lachance 2004). The amplified DNA was cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Roberts Research Institute (London, Ontario, Canada). The sequences were assembled, edited and aligned with the program MEGA5 (Tamura et al. 2011). Phylogenetic placement of the new species was based on a neighbor-joining analysis of D1/D2 LSU rRNA gene sequences with Kimura two-parameter distance transforms. The sequence data consisted of 528 aligned positions. The robustness of the tree was assessed by the bootstrap method with 1,000 iterations.

#### Growth conditions and D-xylose fermentation assays

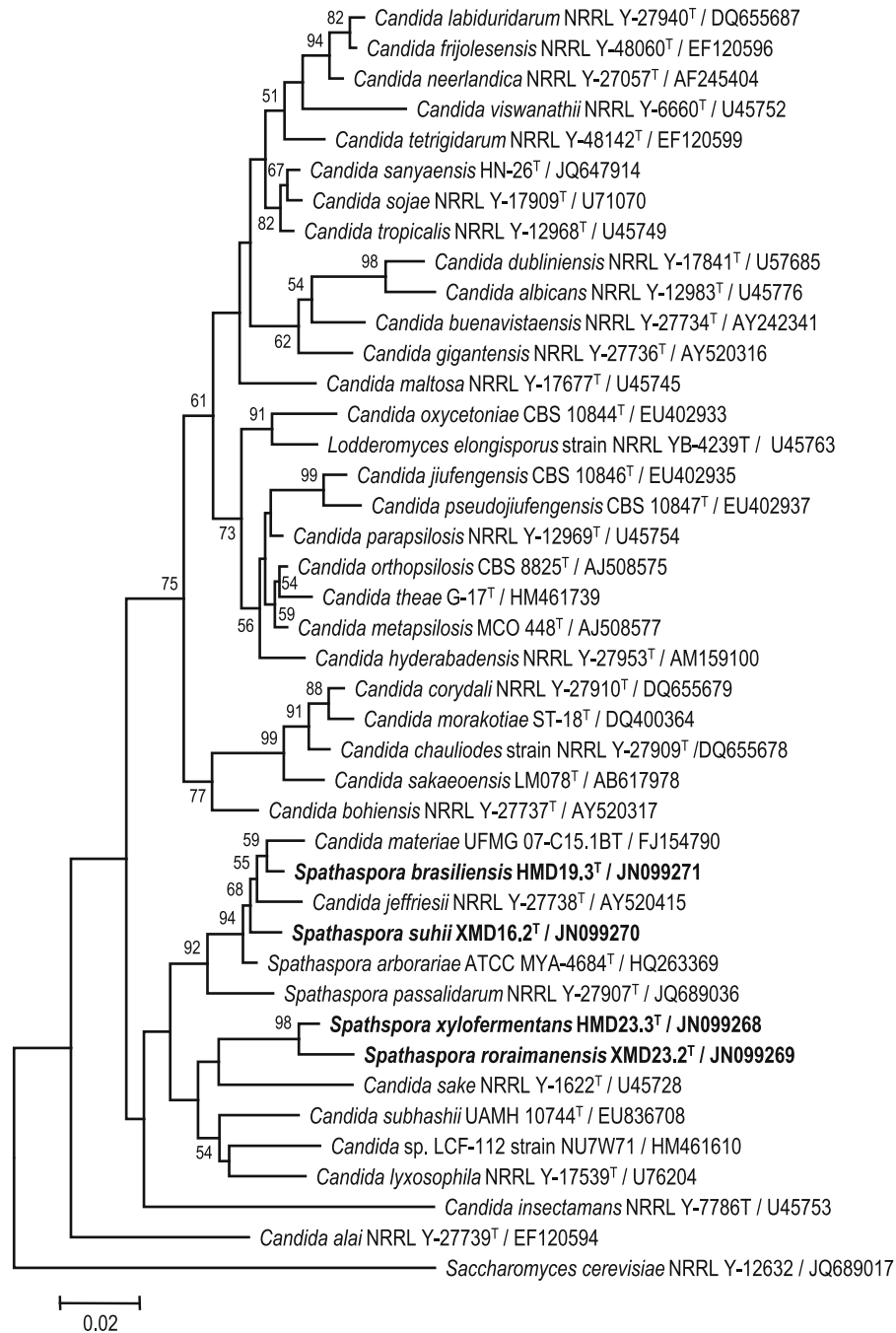
The ability to ferment D-xylose was tested in Durham tubes containing a 2 % (w/v) solution of the sugar (Kurtzman et al. 2011a). The tubes were incubated at 25 °C on an incubator shaker (New Brunswick) at 100 rpm for up to 25 days. *Candida shehatae* CBS 5813, *C. insectosa* CBS 4286, *C. lignosa* CBS 4705 and *Scheffersomyces stipitis* NRRL Y-7124 were used as positive controls for D-xylose fermentation (Cadete et al. 2009). Ethanol production during aerobic batch growth in YP medium (1 % yeast extract and 2 % peptone) adjusted to pH 5.0 with HCl and supplemented with 2 % glucose or xylose was also performed for all isolates of the new species. For this, the cells were grown in YP medium with shaking at 28 °C (160 rpm) in cotton-plugged Erlenmeyer flasks filled to 1/5 of the volume. The inocula for growth assays

were prepared by transferring aseptically a single colony from a plate into 5 mL of the glucose or xylose medium and allowing growth to proceed to stationary phase for 2–3 days. These cells were then used to inoculate fresh media of similar composition at a rate of 1 %. Samples were taken regularly, centrifuged (5,000g, 1 min), and their supernatants used for the determination of sugars and ethanol. Glucose was measured by the glucose oxidase and peroxidase method using a commercial kit (BioDiagnostica-Laborclin, Brazil), and xylose was determined with dinitrosalicylic acid as described by Miller (1959). Ethanol was determined with alcohol oxidase (Sigma, USA) and peroxidase (Toyobo do Brasil, Brazil) as described previously (Alves et al. 2007). Growth was followed by turbidity measurements at 570 nm after appropriate dilution of the medium samples in distilled water. The ethanol yields during growth on glucose ( $Y_{E/ glu}$ , g ethanol g<sup>-1</sup> glucose) or xylose ( $Y_{E/ xyl}$ , g ethanol g<sup>-1</sup> xylose) were calculated taking into account the amount of sugar consumed at the point of maximum ethanol production. Reported values are averages ± mean deviations obtained from independent duplicate cultures, and were analyzed using the paired *t* test.

## Results and discussion

### Species delineation, classification, and ecology

Spore morphology of the three sexual species was typical of the genus *Spathaspora* and neighbor-joining analysis of D1/D2 LSU rRNA gene sequences indicated that the four new species belong to the *Spathaspora* clade (Fig. 1). *Spathaspora brasiliensis* and *S. suhii* belong to the same subclade as do *S. arborariae*, *C. jeffriesii*, and *C. materiae*. Only one isolate of *S. brasiliensis* was isolated in the present study. We describe it based on only a single strain because of its ability to ferment D-xylose, a biotechnological trait of industrial interest for production of biofuels through fermentation of plant hydrolysates. *Spathaspora brasiliensis* UFMG-HMD19.3<sup>T</sup> differs by eight substitutions in the D1/D2 domains and by 13 differences in the ITS region from its least divergent relative, *C. materiae*. The smallest degree of sequence divergence observed for *Spathaspora suhii* UFMG-XMD16.2<sup>T</sup> was seven substitutions and four gaps



**Fig. 1** Phylogenetic placement of *Spathaspora brasiliensis*, *S. roraimanensis*, *S. suhii*, and *S. xylofermentans* inferred by the neighbor-joining analysis of the D1/D2 LSU rRNA gene regions. Bootstrap values (1,000 replicates) of 50 % and above

are shown. Distances were Kimura 2-parameter transformed. A total of 528 positions were used in the analysis, which was conducted with the program MEGA5

from *S. arborariae* in the D1/D2 domains. The ITS and D1/D2 and sequences of the two isolates of *Spathaspora suhii* (UFMG-XMD16.2<sup>T</sup> and UFMG-

HMD16.3) were identical, as were those of the two isolates of *Spathaspora xylofermentans* (UFMG-HMD23.3<sup>T</sup> and UFMG-HMD 25.1). The two are

sister species but differ by 76 changes in the combined ITS region (19 substitutions and 46 gaps) and D1/D2 domains (6 substitutions and 5 gaps) and are well separated phylogenetically from other members of the genus. In fact, the phylogeny presented in Fig. 1 would suggest that *S. roraimanensis* and *S. xylofermentans* are closer relatives of *Candida sake*, *Candida subhashii*, and *Candida lyxosophila*. However, the low bootstrap values obtained for the relevant subclade betray the weak or conflicting phylogenetic signals present in these sequences. This is further corroborated by the observation that the branching order was greatly dependent on taxon sampling (data not shown), which is why we have included all available species of the *Candida albicans* and *Lodderomyces elongisporus* subclades. Furthermore, analysis of the same data by maximum likelihood or maximum parsimony failed to place all known *Spathaspora* species in the same clade. Given the conserved ascospore morphology observed across the species assigned to this genus, it will be of interest to see eventual multi-locus phylogenies. In particular this is to our knowledge the first suggestion that the enigmatic position of *C. sake* (Lachance et al. 2011) may be on its way to being resolved. Last, given the frail nature of the topology depicted in Fig. 1, the utmost circumspection will be called for in implementing the nomenclatural revisions prompted by the forthcoming Melbourne Code (Norvell 2011), which will require the elimination of dual names and a redefinition of genera such as *Lodderomyces*, *Candida*, and even possibly *Spathaspora*.

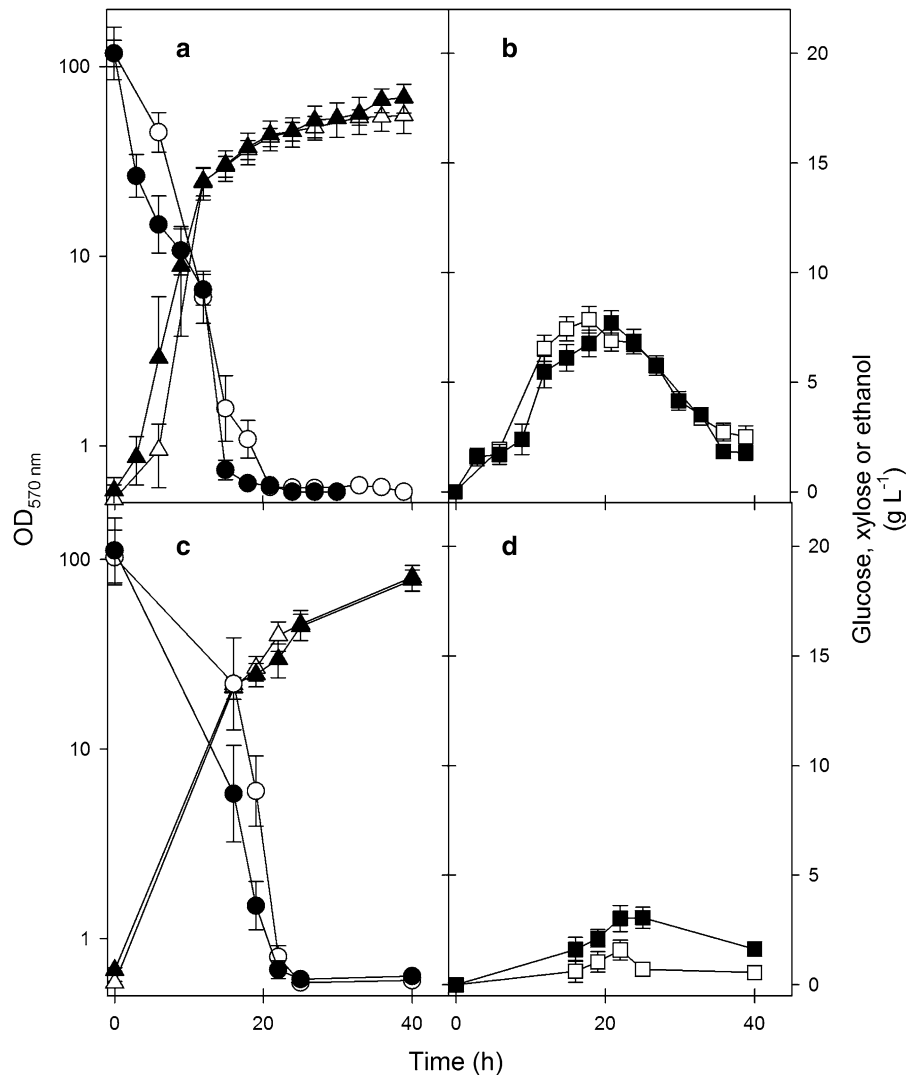
All species of the *Spathaspora* clade were isolated from rotting-wood or insects associated with this substrate (Cadete et al. 2009; Nguyen et al. 2011). *Spathaspora brasiliensis* and *S. suhii* were isolated from rotting-wood samples collected in the ecological reserve of São João da Baliza and *S. roraimanensis* and *S. xylofermentans* were obtained from samples collected in the ecological reserve of Mucajá. All species were minor component of the very diverse yeast communities isolated from rotting wood in these areas (R. M. Cadete and C. A. Rosa, unpublished results).

The new species belonging to the *Spathaspora* clade did not form septate hyphae or pseudohyphae. This morphological characteristic was described as present in *S. passalidarum*, the type species of the genus. Brazilian species belonging to the *Spathaspora* clade (*S. arborariae*, *S. brasiliensis*, *S. suhii*,

*S. xylofermentans*, *S. roraimanensis* and *C. materiae*) be separated from others species based on the assimilation of L-sorbose, which does not occur in the species isolated from other countries (*S. passalidarum*, *C. insectamans* and *C. jeffriesii*). *Spathaspora roraimanensis* and *S. xylofermentans* can be distinguished from all other species based on the ability to grow in the presence of cycloheximide 0.01 %. The former also assimilated L-arabinose, whereas all other species in the clade do not use this sugar. The new species also differ from *S. passalidarum*, *S. arborariae*, and *C. jeffriesii* by failing to assimilate D-ribose. Separation of *S. brasiliensis* from *S. suhii* is difficult as these species had identical growth profiles. In this case, sequencing of D1/D2 domains is recommended to differentiate these species.

### Growth and fermentation of glucose or xylose by the new species

Although initial results with Durham tubes indicated that the new species were unable to ferment xylose, a detailed analysis of glucose and xylose utilization by these yeasts during aerobic batch growth showed that all can produce ethanol from either sugar, albeit with differing efficiencies (Figs. 2, 3). *Spathaspora xylofermentans* grew well on both carbon sources (Fig. 2a, b) and produced ethanol from both sugars ( $Y_{E/\text{glu}} = 0.37 \pm 0.02$ ;  $Y_{E/\text{xy1}} = 0.34 \pm 0.04$ ) at yields similar to those reported for other xylose-fermenting *Spathaspora* yeasts (see Cadete et al. 2009). However, the lower levels of ethanol produced during aerobic growth on glucose or xylose by the other three *Spathaspora* species, *S. brasiliensis* ( $Y_{E/\text{glu}} = 0.08 \pm 0.01$  and  $Y_{E/\text{xy1}} = 0.12 \pm 0.04$ , see Fig. 2c, d), *S. roraimanensis* ( $Y_{E/\text{glu}} = 0.18 \pm 0.04$  and  $Y_{E/\text{xy1}} = 0.29 \pm 0.05$ , see Fig. 3a, b), and *S. suhii* ( $Y_{E/\text{glu}} = 0.11 \pm 0.03$  and  $Y_{E/\text{xy1}} = 0.14 \pm 0.05$ , see Fig. 3c, d), indicated that these yeasts (no significant differences for ethanol production were found among these species), like many other yeast species (Suh et al. 2003; Stambuk et al. 2003; Merico et al. 2007; Barbosa et al. 2009; Boonmak et al. 2011; Rozpędowska et al. 2011), preferentially consume these sugars oxidatively. Although the molecular basis for efficient xylose fermentation is complex and not yet fully understood (Hou 2012; Long et al. 2012), the discovery and analysis of these closely related *Spathaspora*



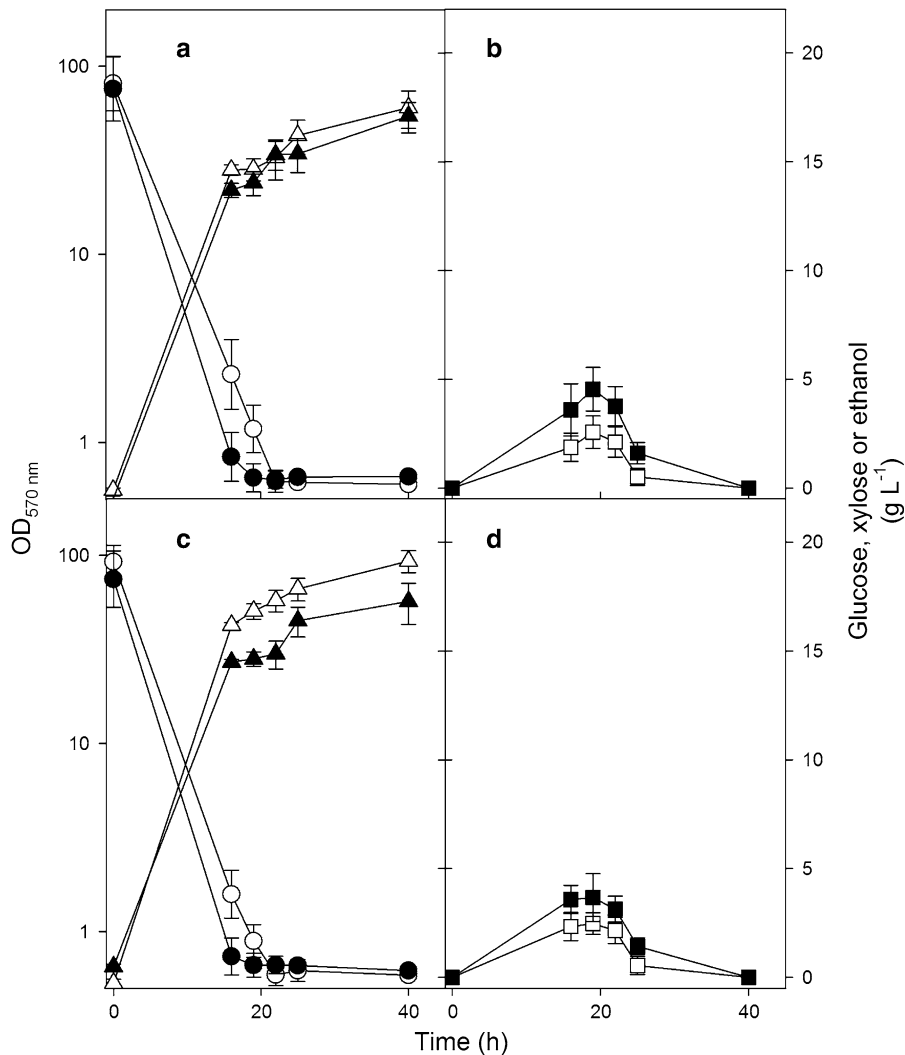
**Fig. 2** Typical aerobic batch growth of *Spathaspora xylofermentans* UFMG-HMD25.1 (**a, b**) and *Spathaspora brasiliensis* UFMG-HMD19.3 (**c, d**) on 20 g L<sup>-1</sup> of glucose (open symbols) or D-xylose (black symbols). Cell growth (triangles), the consumption of sugars (circles), and the production of ethanol

(squares) by *S. xylofermentans* or *S. brasiliensis* were determined during growth in rich YP medium with shaking (160 rpm) at 28 °C. Reported values are averages ± mean deviations obtained from independent duplicate cultures

species capable of xylose fermentation may contribute important genomic information that could be used to improve the efficiency of pentose assimilation by yeasts (Wohlbach et al. 2011), a significant limitation in cellulosic biofuel production. It will be also important to verify whether the new yeast species can be used directly for bioethanol production from lignocellulosic hydrolysates, as has been recently shown for *S. arborariae* (Da Cunha-Pereira et al. 2011).

Description of *Spathaspora brasiliensis* Cadete, Zilli, Vital, Gomes, Stambuk, Lachance and Rosa sp. nov

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–4 μm). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 2 days at 17 °C, colonies are white, butyrous, and glistening. In Dalmau plates after



**Fig. 3** Typical aerobic batch growth of *Spathaspora roraimanensis* UFMG-XMD23.2 (**a, b**) and *Spathaspora suhii* UFMG-XMD16.2 (**c, d**) on 20 g L<sup>-1</sup> of glucose (open symbols) or D-xylose (black symbols). Cell growth (triangles), the consumption of sugars (circles), and the production of ethanol

(squares) by *S. roraimanensis* or *S. suhii* were determined during growth in rich YP medium with shaking (160 rpm) at 28 °C. Reported values are averages ± mean deviations obtained from independent duplicate cultures

2 weeks on cornmeal agar, pseudohyphae are not formed. Sporulation occurs on dilute (1:9 and 1:19) V8 and YCBAS agars at 20 °C after 5 days (Fig. 4). Unconjugated asci are formed from single cells with a single greatly elongated ascospore tapered and curved at the ends. Asci are persistent. Fermentation of glucose, galactose, maltose and trehalose are positive. Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Assimilation of carbon compounds: glucose, galactose,

L-sorbose, sucrose, maltose, cellobiose, trehalose, melizitose, D-xylose, ethanol (slow or negative), glycerol (slow or negative), erythritol, ribitol, D-mannitol, glucitol, salicin, lactic acid, succinic acid, hexadecane, xylitol, ethyl acetate, and N-acetyl-D-glucosamine. No growth occurs on lactose, melibiose, raffinose, inulin, soluble starch, D-arabinose, L-arabinose, D-ribose, L-rhamnose, galactitol, citric acid, myo-inositol, methanol, D-glucosamine, acetone, isopropanol and gluconate. Assimilation of nitrogen compounds: positive for ethylamine-HCl, lysine 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 37 °C is weak. Growth on YM agar with 10 % sodium chloride is negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100 µg cycloheximide mL<sup>-1</sup> the growth is negative. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is rotting wood in Amazonian forest ecosystem, in the state of Roraima, Brazil.

### Type

The type strain accession number of *Spathaspora brasiliensis* is UFMG-HMD19.3<sup>T</sup>. It was isolated from rotting wood in Brazil. It has been deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (Coleção Brasileira de Micro-organismos de Ambiente e Industria, CBMAI), Campinas, São Paulo, Brazil, as strain CBMAI 1425<sup>T</sup> and in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 12679<sup>T</sup>. The Mycobank number is MB 801224.

### Etymology

The epithet *brasiliensis* (bra.si.li.en'sis) L. nom. f. adj. *brasiliensis*, of or pertaining to the country (Brazil) where this yeast was found.

Description of *Spathaspora roraimanensis* Cadete, Zilli, Vital, Gomes, Stambuk, Lachance and Rosa sp. nov

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–4 µm). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 2 days at 17 °C, colonies are white, butyrous, and glistening. In Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae are not formed. Sporulation occurs on dilute (1:19) V8 and YCBAS agars at 20 °C after 5 days (Fig. 4). Unconjugated asci are formed from single cells with a single greatly elongated ascospore tapered and curved at the ends. Asci are not dehiscent. Fermentation of glucose, galactose, maltose and trehalose are positive. Xylose

fermentation in negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Assimilation of carbon compounds: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, D-xylose, L-arabinose (slow), ethanol, erythritol, ribitol, glucitol, salicin, lactic acid, succinic acid, citric acid (variable), hexadecane, xylitol, and N-acetyl-D-glucosamine. No growth occurs on lactose, melibiose, raffinose, melizitose, inulin, soluble starch, D-arabinose, D-ribose, L-rhamnose, glycerol, galactitol, D-mannitol, myo-inositol, methanol, D-glucosamine, acetone, isopropanol, ethyl acetate and gluconate. Assimilation of nitrogen compounds: positive for ethylamine-HCl, lysine 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 37 °C is weak. Growth on YM agar with 10 % sodium chloride is negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100 µg cycloheximide mL<sup>-1</sup> the growth is positive. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is rotting wood in Amazonian forest ecosystem, in the state of Roraima, Brazil.

### Type

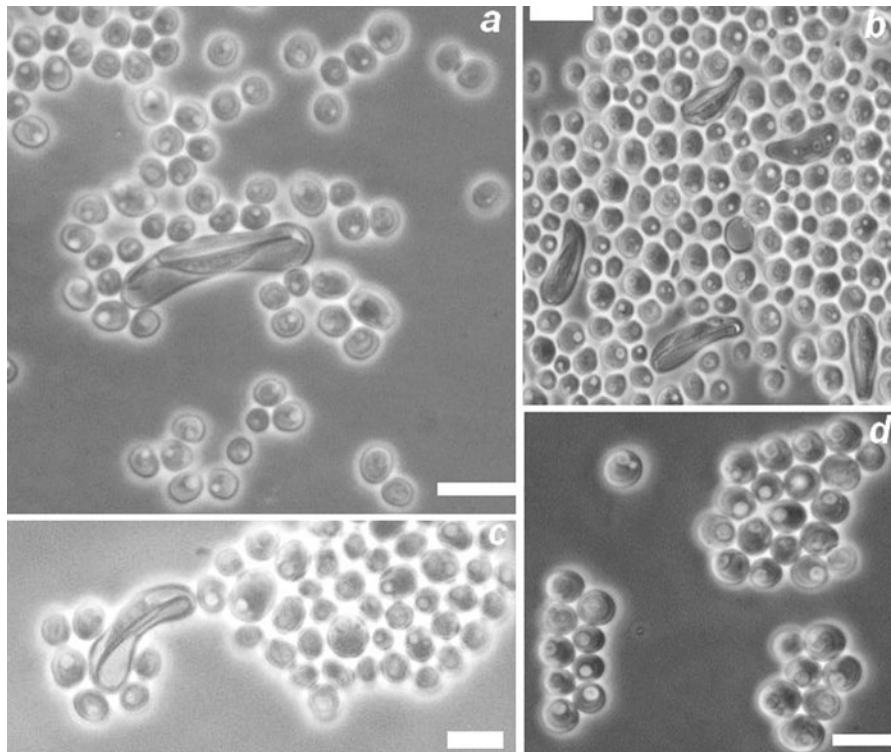
The type strain accession number of *Spathaspora roraimanensis* is UFMG-XMD 23.2<sup>T</sup>. It has been deposited in the Brazilian Collection of Environmental and Industrial Microorganisms, Campinas, São Paulo, Brazil, as strain CBMAI 1427<sup>T</sup> and in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 12681<sup>T</sup>. The Mycobank number is MB 801225.

### Etymology

The epithet *roraimanensis* (ro.ra.i.ma'ne.sis) L. nom. f. adj. *roraimanensis*, of or pertaining to the state in Brazil where this yeast was found.

Description of *Spathaspora suhii* Cadete, Zilli, Vital, Gomes, Stambuk, Lachance, and Rosa sp. nov

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal



**Fig. 4** Budding yeast cells and asci of *Spathaspora brasiliensis* (a) *S. roraimanensis* (b) *S. suhii* (c) and budding cells of *S. xylofermentans* (d) on diluted V8 agar after 5 days at 20 °C. Scale bar = 5  $\mu$ m

(2–3  $\times$  2–4  $\mu$ m). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 2 days at 17 °C, colonies are white, butyrous, and glistening. In Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae are not formed. Sporulation occurs on dilute (1:9) V8 and YCBAS agars at 20 °C after 5 days (Fig. 4). Unconjugated asci are formed from single cells with a single greatly elongated ascospore tapered and curved at the ends. Asci are not dehiscent. Fermentation of glucose, galactose, maltose and trehalose are positive. Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Assimilation of carbon compounds: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melizitose, D-xylose, ethanol (slow), erythritol, ribitol, D-mannitol, glucitol, salicin, lactic acid (variable), succinic acid, hexadecane, xylitol, and *N*-acetyl-D-glucosamine. No growth occurs on lactose, melibiose, raffinose, inulin, soluble starch, D-arabinose, L-arabinose, D-ribose, L-rhamnose, glycerol, galactitol,

citric acid, myo-inositol, methanol, D-glucosamine, acetone, isopropanol, ethyl acetate, and gluconate. Assimilation of nitrogen compounds: positive for ethylamine-HCl, lysine 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 37 °C is positive. Growth on YM agar with 10 % sodium chloride is negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100  $\mu$ g cycloheximide mL<sup>-1</sup> the growth is negative. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is rotting wood in Amazonian forest ecosystem, in the state of Roraima, Brazil.

#### Type

The type strain accession number of *Spathaspora suhii* is UFMG-XMD16.2<sup>T</sup>. It was isolated from rotting wood in Brazil. It has been deposited in the Brazilian Collection of Environmental and Industrial Microorganisms, Campinas, São Paulo, Brazil, as strain

CBMAI 1426<sup>T</sup> and in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 12680<sup>T</sup>. The Mycobank number is MB 801226.

### Etymology

The epithet *suhii* (suh'i.i) L. gen. m. n. *suhii*, referring to Dr. Sung-Oui Suh, in recognition of his contributions to yeast systematics and ecology.

### Description of *Spathaspora xylofermentans*

Cadete, Gomes, Stambuk, Lachance and Rosa sp. nov

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–4 μm). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 2 days at 17 °C, colonies are white, rough, and glistening. In Dalmat plates after 2 weeks on cornmeal agar, pseudohyphae are not formed. Asci or signs of conjugation were not seen on sporulation media (Fig. 4). Fermentation of glucose, maltose and trehalose are positive. Xylose fermentation in negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Assimilation of carbon compounds: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melizitose, D-xylose, ethanol (slow), glycerol (variable), erythritol, ribitol, glucitol, salicin, lactic acid, succinic acid, citric acid (variable), hexadecane, xylitol, and *N*-acetyl-D-glucosamine. No growth occurs on lactose, melibiose, raffinose, inulin, soluble starch, D-arabinose, L-arabinose, D-ribose, L-rhamnose, galactitol, D-mannitol, myo-inositol, methanol, D-glucosamine, acetone, isopropanol, ethyl acetate, and glucante. Assimilation of nitrogen compounds: positive for ethylamine-HCl, lysine 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 37 °C is very weak. Growth on YM agar with 10 % sodium chloride is negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100 μg cycloheximide mL<sup>-1</sup> the growth is positive. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is rotting wood in

Amazonian forest ecosystem, in the state of Roraima, Brazil.

### Type

The type strain accession number of *Spathaspora xylofermentans* is UFMG-HMD23.3<sup>T</sup>. It was isolated from rotting wood in Brazil. It has been deposited in the Brazilian Collection of Environmental and Industrial Microorganisms, Campinas, São Paulo, Brazil, as strain CBMAI 1428<sup>T</sup> and in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 12682<sup>T</sup>. The Mycobank number is MB 801227.

### Etymology

The epithet *xylofermentans* (xy.lo.fer.men'tans) L. nom. f. adj. *xylofermentans*, pertaining to the ability of this yeast to ferment D-xylose.

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**Conflict of interest** None.

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