Carlos Rosa ORCID iD: 0000-0002-0056-9075

Gisele de Fatima Leite Souza ORCID iD: 0000-0001-8995-9485

Chris Hittinger ORCID iD: 0000-0001-5088-7461

The Brazilian Amazonian rainforest harbors a high diversity of yeasts associated with rotting wood, including many candidates for new yeast species

Katharina O. Barros[1,2,3], Flávia B. M. Alvarenga[1], Giulia Magni[1], Gisele F. L. Souza[1], Maxwel A. Abegg[4], Fernanda Palladino[1], Sílvio S. da Silva[4], Rita C. L. B. Rodrigues[4], Trey K. Sato[2], Chris Todd Hittinger[2,3], Carlos A. Rosa[1*]

1 Departmento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil.

2 DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI 53726, USA.

3 Laboratory of Genetics, J. F. Crow Institute for the Study of Evolution, Wisconsin Energy Institute, Center for Genomic Science Innovation, University of Wisconsin-Madison, Madison, WI 53726, USA.

4 Institute of Exact Sciences and Technology (ICET), Federal University of Amazonas (UFAM), Itacoatiara, AM, 69100-000, Brazil.

5 Department of Biotechnology, Engineering School of Lorena, University of São Paulo, Estrada Municipal do Campinho s/n, Lorena, SP, 12602-810, Brazil.

Abstract

This study investigated the diversity of yeast species associated with rotting wood in Brazilian Amazonian rainforests. A total of 569 yeast strains were isolated from rotting wood samples collected in three Amazonian areas (Universidade Federal do Amazonas-UFAM, Piquiá, and Carú) in the municipality of Itacoatiara, Amazon state. The samples were cultured in yeast nitrogen base (YNB)-D-xylose, YNB-xylan, and sugarcane bagasse and corncob hemicellulosic hydrolysates (undiluted and diluted 1:2 and 1:5).

Sugiyamaella was the most prevalent genus identified in this work, followed by *Kazachstania*. The most frequently isolated yeast species were *Schwanniomyces polymorphus*, *Scheffersomyces amazonensis*, and *Wickerhamomyces* sp., respectively. The alpha diversity analyses showed that the dryland forest of UFAM was the most diverse area, while the floodplain forest of Carú was the least. Additionally, the difference in diversity between UFAM and Carú was the highest among the comparisons. Thirty candidates for new yeast species were obtained, representing 36% of the species identified and totaling 101 isolates. Among them were species belonging to the clades *Spathaspora*, *Scheffersomyces*, and *Sugiyamaella*, which are recognized as genera with natural xylose-fermenting yeasts that are often studied for biotechnological and ecological purposes. The results of this work showed that rotting wood collected from the Amazonian rainforest is a tremendous source of diverse yeasts, including candidates for new species.

Graphical Abstract Text

To explore the diversity of the Amazonian rainforest by targeting the group of yeasts with the ability to assimilate lignocellulosic sugars, the authors collected rotting wood samples in three sites of the forest, including both drylands and floodplain areas. Dryland areas in the Brazilian Amazonian rainforest has higher diversity of yeasts associated with rooting wood than floodplain areas. Also, a high number of candidates for novel species is present in rotting wood samples.

Take Away

- Rotting wood is a great source of yeast species that can assimilate lignocellulosic sugars.
- Dryland areas in the Brazilian Amazonian rainforest has higher diversity of culturable yeasts associated with rooting wood than floodplain areas.
- A great number of candidates for novel species is present in rotting wood samples.
- UFAM and YNB-xylose were the area and medium, respectively, in which the highest number of new species candidates were recovered.

Correspondence:
Carlos A. Rosa
carlrosa@icb.ufmg.br

Introduction

Yeast species are present in live and decaying plant parts, and they have beneficial interactions with insects (Cadete et al. 2017). While the yeasts provide nutrients to the insects by contributing to food digestion, the insects carry the yeasts to different habitats (Stefanini et al., 2018). The production of volatile metabolites (i.e., acetate esters) by yeasts attracts insects, who have efficient olfactory receptors adapted to yeast fermentation products that signal substrates for adult and larval feeding (Ljunggren et al., 2019; Becher et al. 2018; Arguello et al., 2013). Several studies described the vectoring of yeasts by insects. For example, fermentation can increase the attractiveness of natural substrates toward *Drosophila* (Christiaens et al., 2014), and *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Issatchenkia terricola*, and *Pichia kluyveri* are some examples of yeast species isolated from adults and larvae of *Drosophila* (Lewis and Hamby, 2019; Bellutti et al., 2018; Hamby and Becher, 2016). Yeasts from the *Metschnikowia* clade seem to have evolved in co-specific associations with nitidulid beetles of the genus *Conotelus* that vector yeasts among flowers, where the yeasts grow in nectar and provide a rich diet for the beetle larvae (Santos et al., 2020; Guzmán et al., 2013). *Starmerella* species are prevalent in stingless bees in Brazilian savannas, and *Torulaspora delbrueckii* was also frequently isolated from those insects (da Costa-Neto and Morais, 2020). Finally, the guts of wood-feeding insects are rich in ascomycete yeasts, in particular xylose-fermenting and xylose-assimilating yeasts, which are commonly present in the guts, frass, and larvae of beetles of Passalidae and Cerambycidae families (Barros et al. 2021, Souza et al., 2017; Urbina et al., 2013; Nguyen et al., 2006).

Several yeast species (e.g. species of *Spathaspora*, *Scheffersomyces,* and *Sugiyamaella* clades) found in the guts and frass of wood-boring insects were also found in their habitats, including rotting wood (Shi et al., 2021; Lopes et al., 2018a; Nguyen et al., 2006). Yeasts found in decomposing wood have been shown to often have the ability to assimilate and/or ferment lignocellulosic sugars, especially pentoses from the hemicellulosic fraction (Barros et al., 2020). As lignocellulose is an abundant raw material found in nature and is a major agricultural waste product (Faostat, 2017), those yeasts have been studied for their capacity to generate value-added fuels and products (e.g., ethanol, xylitol, and xylanases) from lignocellulosic materials (Kumar et al., 2021).

Brazilian Amazonia corresponds to 58.9% of Brazilian territory, extending along the North region of the country (IBGE, 2020). Amazonia is not only the world's most diverse rainforest, but is also the region in tropical America that has contributed most to its total biodiversity (Antonelli et al., 2018). This biome is classified as a biodiversity hotspot; biodiversity hotspots are areas characterized by outstanding concentrations of endemic species and anthropogenic pressure (Myers et al., 2000). Although the term is related mostly to plants, it can be extended, for example, to invertebrates and yeasts, because there are many insect-yeast-plant interactions (Péter et al., 2017; Myers et al., 2000). The first study to investigate the Brazilian Amazonian Forest with the goal of isolating and identifying new D-xylose-fermenting yeasts associated with rotting wood was conducted by Cadete et al. (2012). A total of 224 yeast strains were isolated from 40 rotting wood samples collected in different areas of the forest. Since then, other studies have been carried out with the goal of isolating cellobiose-fermenting (Lopes et al. 2018a) or xylose-fermenting yeasts (Souza et al., 2017).

Despite the success of those previous collections in the Brazilian Amazonia, Cadete et al. (2012), Lopes et al. (2018a), and Souza et al. (2017) used minimal media or yeast extract malt to isolate the species. Only a single study of Atlantic Rain Forest sites has collected yeasts using sugarcane bagasse hydrolysate as the isolation medium (Morais et al. 2020). Deconstruction of sugarcane bagasse by acid hydrolysis results in the production of toxic compounds, including acetic acid, furan derivatives, and phenolic compounds (Liu et al., 2021). These compounds are known to inhibit yeast growth and fermentation (Sato et al., 2016), so selection and isolation of yeasts with this type of medium might identify species with relevance to the lignocellulosic biofuel industry. To explore the diversity of the Amazonian rainforest by targeting the group of yeasts with the ability to assimilate lignocellulosic sugars, we collected rotting wood samples in three sites of the forest, including both drylands and floodplain areas. To isolate the maximum number of species, undiluted and diluted hemicellulosic hydrolysates from different raw materials, as well as minimal medium with xylose or xylan, were used as isolation media.

Methods

Yeast isolation and identification

Yeasts were isolated from rotting wood samples of unidentified trees collected in three areas of the Brazilian Amazonian rainforest in the Amazon state. The areas were Campus II of Universidade Federal do Amazonas (UFAM) (03° 05.654' S, 58° 27.464' W), Piquiá Sol Nascente Community (03° 01.045' S, 58° 28.830' W), and Carú (03° 35.923' S, 58° 44.905' W). They are located in the municipality of Itacoatiara, which is 270 km east of Manaus, the capital of the Amazon state. The Amazon state presents a tropical climate, where the average annual temperature is between 25.5 and 27.5°C, and the annual

precipitation ranges between 1,500 and 2,100 mm, with rain concentrated in the summer months and a humid season throughout the year (Alves et al., 2019). While Carú is a floodplain forest, known as a várzea, UFAM and Piquiá are dryland forests (terra firme) characterized by dense ombrophilous forests. Várzea forests are annually flooded by white water (nutrient-rich water) with consequent enrichment of the soil (Parolin et al., 2004). Twenty samples of rotting wood from the trunks of the trees were collected in each area in February 2019. The samples were stored in sterile plastic bags and transported under refrigeration to the laboratory immediately after the collecting. For each sample, 0.5 g was placed separately in flasks with 10 mL sterile sugarcane bagasse and corncob hemicellulosic hydrolysate at three different concentrations (undiluted, diluted 1:2, and diluted 1:5 with sterile water), without nutritional supplementation (the protocol of preparation of these hydrolysates is described below). The samples were also inoculated in the culture media YNB-xylose (0.67% yeast nitrogen base, 2% xylose) and YNB-xylan (0.67% yeast nitrogen base, 2% xylan). The tubes were incubated at 30ºC on an orbital shaker (New Brunswick, USA) at 200 rpm until the growth was detected. Then, 0.5 mL of each culture was transferred to tubes containing 5 mL of sugarcane bagasse hydrolysate in the same concentrations listed previously, and the tubes were incubated as described above. Thus, all isolated yeasts were exposed to at least one round of growth in sugarcane bagasse hydrolysate. After yeast growth, one loopful of culture from each tube was streaked on YM agar (glucose 1 %, yeast extract 0.3 %, malt extract 0.3 %, peptone 0.5 %, agar 2 %, and chloramphenicol 0.02 %) (Sena et al., 2017). The plates were incubated at 25ºC until the yeast colonies developed. The different yeast morphotypes were purified by repeated streaking on YM agar plates and preserved at -80ºC for later

identification. The yeasts were characterized physiologically and morphologically using standard methods (Kurtzman et al., 2011).

Isolates with identical morphological and physiological characteristics were grouped together and subjected to microsatellite-primed $(GTG)_5$ PCR fingerprinting (Libkind et al., 2003). Yeast strains with identical PCR fingerprinting patterns were grouped and putatively considered to belong to the same species (Sampaio et al., 2001; Gomes et al., 2015; Lopes et al., 2018b). At least half of the yeast isolates of each molecular group were identified by sequencing of the ITS-5.8S region and the D1/D2 variable domains of the large subunit rRNA gene. Species identifications were performed by analysis of the sequences of the ITS-5.8S region and the D1/D2 variable domains (White et al., 1990; O'Donnell, 1993; Kurtzman and Robnett, 1998; Lachance et al., 1999). The amplified DNA was cleaned and sequenced in an ABI 3130 Genetic Analyzer automated sequencing system (Life Technologies, California, USA) using BigDye v3.1 and POP7 polymer. The sequences were assembled, edited, and aligned with the program MEGA7 (Kumar et al., 2016). They were compared with annotated yeast sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST at http://www.ncbi.nlm.nih.gov/) (Sayers et al., 2022).

Phylogenetic analyses

Phylogenetic analyses were made for possible new species among the major clades of yeasts associated with rotting wood habitats. Phylogenetic placements of the candidates for novel species from the clades *Spathaspora* and *Sugiyamaella/Spencermartinsiella* were based on a maximum-likelihood analyses of D1/D2 sequences totaling 532 and 533 aligned positions, respectively. The evolutionary distances were computed using the

Tamura-Nei model and a gamma-distributed substitution rate within invariant sites. Using the alignment as input file, we selected the best substitution models based on the lowest AIC score generated by the software PhyML 3.0 (Guindon et al., 2010). Bootstrap values were determined from 1000 iterations. For members belonging to the genus *Cyberlindnera*, the phylogenetic placement was based on neighbor-joining analysis of D1/D2 sequences with 531 aligned positions and 1000 pseudoreplicates.

Hemicellulosic hydrolysates

Both sugarcane bagasse hemicellulosic hydrolysate (SBHH) and corncob hemicellulosic hydrolysate (CHH) were obtained by acid hydrolysis in a bioreactor under the following conditions: 121°C during 20 min; sulfuric acid 98% with a ratio of 1:10 (100mg H_2SO_4 per gram of sugarcane bagasse or corncob). The liquid fraction was recovered by vacuum filtration, the pH was adjusted to 5.5 with calcium oxide (CaO), and it was treated with 2.4% w/v activated vegetable charcoal in the form of refined powder. Then, the hydrolysates were autoclaved at 111°C for 15 min. No detoxification step was performed. Concentrations of sugars and acetic acid were determined by high-performance liquid chromatography - HPLC (Shimadzu, Kyoto, Japan), using the following conditions: Supelco Analytical C-610 H column (Sigma-Aldrich, USA), maintained at 45°C; volume injection of 20µL; refractive index detector RID 10-A (Shimadzu, Kyoto, Japan); 5 mM H_2SO_4 mobile phase as eluent at a flow rate of 0.6 mL min^{-1}. Furfural and hydroxymethylfurfural were measured by HPLC using a dual k absorbance detector in a 276 nm wavelength (Waters 2487, Milford-MA-USA) and Waters Resolve 5 µL Spherical C18 column (3.9 x 300 mm) at 25°C; solution of acetonitrile–water 1:8 (v/v) with 1% of acetic acid as eluent, at a flow rate of 0.8 mL/min and injection volume of 20

μL (Rodrigues et al., 2010). The compositions of the hydrolysates were: 0.004% 5-HMF, 0.03% furfural, 0.5% acetic acid, 0.6 glucose, and 9% xylose for CHH; 0.013% 5-HMF, 0.02% furfural, 0.5% acetic acid, 0.2% glucose, and 2% xylose for SBHH.

Diversity analyses

To assess the relative abundance and visualize species richness and species evenness of each collection site, we constructed rank abundance curves (RACs) and an accumulation species-richness curve. First, we calculated the rank abundance distribution for each area, which presents an abundance of each species in the sample from the most common (rank one) to the least abundant (highest rank). We fit a model for this distribution, which was chosen from specific models by its AIC (Akaike Information Criterion) in the package *vegan* using the function "radfit". When this function is used, five models are tested simultaneously (Null, Lognormal, Mendelbrot, Preemption, and Zipf), and only a curve of the best model is presented in the graph (Oksanen et al., 2020). Then, we constructed a cumulative richness curve for each location and medium, representing how many species were found in each sample. The line shows the average number of species, while the colored bands are the standard deviations. If the curve of an area is above the curve of another one, the richness in this area is greater than the other. A rarefaction curve was constructed by randomly resampling the pool of N samples several times and then plotting the average number of species found on each sample. The curve initially grows rapidly as the most common species are found and then slightly flattens as the rarest species remain to be sampled. Alpha-diversity analysis was calculated in R version v3.6.3 using the RStudio v1.3.1073 platform and the package *vegan* (Oksanen et al., 2020). In addition, beta-diversity analysis was conducted based on Sørensen dissimilarity, which

studies beta diversity of pairs of sites. This analysis was performed among the different collection sites based on the yeast species using the R packages *vegan* and *ape* (Paradis and Schliep, 2019).

Results and Discussion

Rotting wood is a source of species that can assimilate lignocellulosic sugars and undiscovered candidate species

A total of 569 yeast isolates were obtained from 60 rotting wood samples of three areas of the Amazonian Rainforest in Brazil. Two hundred thirty-five were obtained from samples collected in the Piquiá area, 219 from UFAM, and 115 from Carú. Considering the culture media, 128 strains were isolated in YNB-xylan, 118 in YNB-xylose, 45 in undiluted SBHH, 68 in SBHH diluted 1:2, 78 in SBHH diluted 1:5, 18 in undiluted CCHH, 46 in CCHH diluted 1:2, and 68 in CCHH diluted 1:5 (Fig. 1). The D1/D2 domains and ITS regions of 336 isolates were sequenced, and 83 species were identified, of which 53 were previously known, and 30 represented candidates for novel species. Twenty-eight species were represented by a single isolate, and 55 species were isolated more than once. These species are shown in Table S1, which shows the occurrence and number of each species by isolation site and culture medium. Ascomycetous yeasts were prevalent. The considerable number of identified species, including undescribed species, is likely due to the greater number of media used during the isolation and possibly due to the geographically distinct regions used as sampling sites, both of which are expected to increase the probability of obtaining a higher diversity of species.

Sugiyamaella was the most prevalent genus identified in this study, represented by eleven species (*Su. bahiana*, *Su. paludigena*, *Su. smithiae*, *Su. valenteae*, *Su.*

xylolytica, and six candidates for novel species), followed by *Kazachstania* with eight species (*K. kunashirensis, K. martiniae, K. serrabonitensis, K. wufongensis*, and four candidates for new species). Table 1 shows the candidates for novel species, their most closely related species, and the percent sequence identity. *Sugiyamaella* strains were isolated from all the collection areas in the different culture media utilized, including the hydrolysates. *Kazachstania* strains were recovered mainly in hydrolysates (both diluted and undiluted) from samples from UFAM and Piquiá. Only two species (*Kazachstania* sp. 1 and *K. kunashirensis*), an isolate of each one, came from YNB-xylose. The species of this genus are ubiquitous (Jacques et al., 2016) and have been found previously in sourdoughs (Urien et al., 2019), soil (Lee et al., 2009), the guts of passalid beetles (Suh and Zhou, 2011), rotting wood (Jacques et al., 2016), gastrointestinal tract infections (Alvarez-Perez et al., 2012), and water reservoirs of bromeliads (Araújo et al., 2012). *Kazachstania* sp. 1 and *Kazachstania* sp. 2 are phylogenetically closest to *K. martiniae* and *K. yasuniensis*, respectively. *Kazachstania* sp. 3 and sp. 4 are closest to *K. africana*, and *K. servazzii*, respectively.

Since *Sugiyamaella* species are able to convert D-xylose into ethanol and xylitol (Morais et al., 2013) and produce xylanases (Morais et al., 2020; Lara et al., 2014), they are interesting for biotechnological purposes. Members of this clade are associated with wood-boring insects, frass and habitats of insects, and decaying plant materials (Cadete et al., 2017). Among the three known *Sugiyamaella* species isolated in this study, three (*Su. bahiana*, *Su. valenteae*, and *Su. xylolytica*) were described by Sena et al. (2017). In that study, they found seven new species of the genus in rotting wood samples collected in different regions of Brazil. The new species of *Sugiyamaella* are related to *Su.*

carassensis, *Su. castrensis, Su. paludigena*, *Su. lignohabitans*, *Su. bonitensis, Su. trypani*, and *Su. ayubii*. At the moment, the genus accommodates 33 described species, including 13 that were transferred from the genus *Candida* (Chai et al., 2022; Shi et al., 2021; Urbina et al., 2013).

When phenotypic characteristics were used as the main criteria to classify yeasts prior to the adoption of the one fungus-one name rule, many morphologically indistinguishable species were grouped as *Candida*. Since then, several taxonomic changes have been made based on DNA analysis (Daniel et al., 2014). We isolated 26 species assigned as *Candida*, but they belong to six different clades (*C. albicans, C. blattae, C. maltosa*, *C. orthopsilosis*, *C. tropicalis* - *Lodderomyces/Candida albicans* clade; *C. boidinii* - *Ogataea* clade*; C. boleticola*, *C. natalensis*, - *Kurtzmaniella* clade*; C. ghanaensis, C. gorgasii*, *C. jaroonii*, *C. michaelii* - *Yamadazyma* clade*; C. intermedia*, *C. pseudointermedia* - *Clavispora* clade*; C. palmioleophila* - *Candida glaebosa* clade; *C. melibiosica*, *C. saopaulonensis* - *Metschnikowia* clade; and 10 candidates for novel species). *C. melibiosica* (11 isolates) and *C. tropicalis* (12) were the most frequent species among them. *C. melibiosica* has been used to produce xylitol and arabitol, and it is associated with soil and rotting wood (Han et al., 2019, Morais et al., 2013). *C. intermedia* was isolated from decaying wood, produces xylanase activity, and can ferment xylose (Lara et al., 2014).

Several sequences of the D1/D2 domain and ITS region of the candidates for novel species were similarly related to more than one genus. To be conservative, their sequences were deposited as *Candida* sp. in GenBank. From *Candida* sp. 1 to *Candida* sp. 6, the most closely related described species are *C. tammaniensis* and *C.*

cerambycidarum (*Yamadazyma* clade), *C. sanyaensis* and *C. sojae* (*Lodderomyces/Candida albicans* clade), *C. nivariensis* (*Nakaseomyces* clade), *C. insectorum* (*Yamadazyma* clade), *Torulaspora globosa* and *Ogataea parapolymorpha*, and *Saccharomyces eubayanus* and *Vanderwaltozyma yarrowii*, respectively (Table 1).

Schwanniomyces polymorphus (20 isolates), *Scheffersomyces amazonensis* (16 isolates), *Wickerhamomyces* sp. (16 isolates), *Sugiyamaella* sp. 1 (14 isolates), and *K. serrabonitensis* (13 isolates) were the species most frequently isolated. Soil has been suggested as the natural habitat of *Schw. polymorphus* (Suzuki and Kurtzman, 2010). Previously, this was the most frequently isolated species from rotting wood samples collected at Atlantic rainforest in Brazil in YNB-xylan and YNB-xylose (Morais et al., 2013). This species is also associated with the infrabuccal pocket of the carpenter ant *Camponotus vicinus* (Hymenoptera: Formicidae), but its role in ant development is not clear (Mankowski et al., 2021). While *Schw. polymorphus* was found in all the areas, it was isolated mainly in YNB-xylan and YNB-xylose. Fifteen isolates of *Sch. amazonensis* were obtained nearly exclusively from UFAM in all the media except for undiluted hydrolysates; a single isolate was obtained from Carú in SBHH diluted to 1:5. Species belonging to the *Scheffersomyces* clade are commonly found in rotting wood samples, which suggests they are adapted to this substrate (Kurtzman, 2011). Previous studies isolated *Sch. amazonensis*, first classified as *C. amazonensis*, from rotting wood sampled in a Brazilian Amazonian rainforest (Cadete et al., 2012) and Atlantic rainforest (Lopes et al., 2018b). Thus, rotting wood could be a habitat of *Sch. amazonensis*. Consistent with this potential niche, it ferments xylose and cellobiose well, producing mainly xylitol and ethanol as end-products (Lopes et al., 2018b, Cadete et al., 2016a). *Wickerhamomyces* sp.

strains were isolated from UFAM and Carú samples in both hydrolysate types with and without dilution. This was the only species isolated in undiluted CCHH from UFAM and Carú samples. This species is closely related to *W. edaphicus*, which has been previously isolated from soil samples collected in Thailand, and it has been reported that this species is able to grow in a media containing 60% glucose and 10% NaCl (Limtong et al., 2009). Considering the high concentration of sugars and the mix of inhibitors in CCHH hydrolysate, it is possible that *Wickerhamomyces* sp. is adapted to growing in high osmolarity and adverse environments.

Likewise, *Rhodotorula mucilaginosa* (11 isolates) and *Apiotrichum mycotoxinivorans* (9 isolates) had a significant number of individuals. *Rh. mucilaginosa* isolates were obtained from samples from all the collection sites, and *A. mycotoxinivorans* was isolated from UFAM and Carú. Although *Rh. mucilaginosa* can be associated with human diseases (Ioannou et al., 2019), this ubiquitous basidiomycetous yeast is found in a wide range of aquatic and terrestrial habitats, such as freshwater lakes, ocean, and living and decaying plant parts (Sampaio, 2011). Biotechnological applications for this yeast have been proposed that include carotenoid (Cheng and Yang, 2016; Libkind et al., 2004) and biopolymer production (Hamidi et al., 2020). *A. mycotoxinivorans* is a basidiomycetous yeast used as a microbial feed additive to protect against mycotoxins (Sun et al., 2020). It has been found in rotting wood and water samples and has xylanase and amylase activities (Carvalho et al., 2021; Carla et al., 2014).

Twenty-eight species were represented by single isolates. Among them, 16 have been described previously (*C. albicans*, C. *(Yamadazyma) michaelii*, *C. (Yamadazyma)*

jaroonii, *C. (Yamadazyma) michaelii*, *C.* (Metschnikowiaceae) *saopaulonensis*, *C. tropicalis*, *Saitozyma flava*, *Debaryomyces hansenii*, *D. nepalensis*, *Meyerozyma caribbica*, *M. guilliermondii*, *Rh. alborubescens*, *Saccharomyces cerevisiae*, *Sch. shehatae*, *Su. bahiana*, and *Su. valenteae*). Eleven of the singletons are candidates for new species (*Candida* sp. 1, *Candida* sp. 2, *Candida* sp. 4, Candida sp. 6, *Cyberlindnera* sp. 1, *Cyberlindnera* sp. 2, *Kazachstania* sp. 4, *Kurtzmaniella* sp., *Spathaspora* sp. 2, *Sugiyamaella* sp. 5, and *Torulaspora* sp.).

Some single isolates might represent transient species within the sampling sites. Indeed, studies in which *S. cerevisiae* strains were isolated from rotting wood showed that they were present in extremely low numbers compared to the dominant yeast species (Morais et al., 2020; Hui et al., 2013). *S. cerevisiae* is instead thought to be associated with bark, exudates, leaves, and soil (Hittinger, 2013). In the case of *S. cerevisiae* and other members of the genus, the isolation from those environments has generally been done using different enrichment media (Spurley et al. 2022; Sylvester et al. 2015; Sampaio and Gonçalves, 2008). *M. caribbica* and *M. guilliermondii* are widely distributed in nature, and they also have been found on decaying plant tissues (Ali et al., 2017; Lopes et al., 2018b; Morais et al., 2020). Similarly, *C. albicans* is known as a human commensal and opportunistic pathogen, but it is sporadically found on plant materials (Opulente et al. 2019; Bensasson et al. 2018; Lachance et al., 2011). *Cyberlindnera* species are frequently recovered from this type of substrate (Barros et al., 2021; Boontham et al., 2017). In this study, we found two new species belonging to this clade. *Cyberlindnera* sp. 1 is related to *Cy. japonica* and *Cy. easanensis,* and *Cyberlindnera* sp. 2 is closely related to *Cy. maritima* (Table 1).

Two candidates for new species belonging to *Spathaspora* and one of *Scheffersomyces* were also isolated. *Sp. boniae*, *Sp. arborariae*, and *Scheffersomyces* sp. UFMG-CM-Y365 are the most closely related species, respectively. *Spathaspora* and *Scheffersomyces* are remarkable genera with several species able to ferment xylose and produce great amounts of ethanol (Nakanishi et al., 2017). They have been found in the guts, frass, and larvae of wood-boring insects, as well as in their habitats (Lopes et al., 2016; Urbina and Blackwell, 2012; Nguyen et al., 2006). Although we isolated some described species belonging to the genus *Scheffersomyces*, none of the known *Spathaspora* species were found in this work.

Putative ecological role and biotechnological applications of yeasts isolated from decaying wood

Yeasts benefit insects by providing them with amino acids, vitamins, sterols, and detoxification of plant metabolites. The advantage for the yeasts is a stable environment with a dependable food source and transportation to a new habitat since they are sessile microorganisms (Ljunggren et al., 2019). The guts of saproxylic insects from different families are especially rich in yeasts that carry out the fermentation of the wood-related sugars cellobiose and xylose (Urbina et al., 2013b). Several yeasts isolated in our work, such as *Cyberlindnera*, *Scheffersomyces*, *Spathaspora*, and *Sugiyamaella* species, were previously recovered from wood-boring beetles, and they were reported to ferment lignocellulosic related-sugars. For example, species belonging to the *Sugiyamaella* clade, the most frequent genus in this study, were reported to assimilate/ferment xylose and/or hydrolyze xylan (Morais et al., 2020; Handel et al., 2016; Lara et al., 2014). Because of

these traits, they are suspected to play an important role in the digestion of wood within the beetle gut (Seibold et al. 2019).

Yeasts with ability to assimilate lignocellulosic sugars, especially xylose, and/or hydrolytic enzymes are present in both the initial and advanced stages of wood decay (Cadete et al., 2017). Xylose metabolism is an important trait for industrial purposes, but it is not found in *S. cerevisiae* (Su et al. 2020). The highest fermentation rates were seen in species of the genera *Scheffersomyces* and *Spathaspora* (Cadete et al., 2016b; Urbina and Blackwell, 2012). *Sch. amazonensis*, the second species most frequently isolated in this study, ferments xylose and accumulates high yields of xylitol (Cadete et al., 2016a), which is a polyalcohol classified as a sweetener capable of replacing sucrose (Palladino et al., 2021). In the context of energy, *Sch. stipitis*, *Sch. shehatae* (both present in our samples), and some members of the genus *Spathaspora* are considered prominent species for the conversion of xylose to ethanol (Morais et al., 2020; Cadete and Rosa, 2018; Cadete et al., 2016). Among other species we reported here, *C. tropicalis*, *C. intermedia*, *C. maltosa*, and species of the genera *Cyberlindnera*, *Sugiyamaella*, *Wickerhamomyces*, *Meyerozyma*, and *Pichia* were also reported to ferment xylose into ethanol and/or xylitol (Barros et al., 2021; Narisetty et al., 2021; Bonnato et al., 2020; Geijer et al., 2020; Morais et al., 2020; Bazoti et al., 2017; Sena et al., 2017).

Many yeast species are known for their enzyme production, and several of them were isolated in this study. *C. tropicalis*, *C. intermedia*, *M. guilliermondii*, the basidiomycetous *A. mycotoxinivorans*, and species of the genera *Sugiyamaella*, *Scheffersomyces*, *Spencermartinsiella*, *Rhodotorula*, and *Wickerhamomyces* showed enzyme activity for xylanase and β-xylosidase (Morais et al., 2020; Moreira, 2016; Lara

et al. 2014; Morais et al., 2013). Xylanolytic enzymes are valuable for applications in the pulp and paper industry, as well as the textile and food sectors. Moreover, those enzymes can be used in the hydrolysis of lignocellulosic biomass to produce biofuels and/or biochemicals (Bhardwaj et al., 2019; Lara et al., 2014).

Dryland forests presented higher diversity than floodplain forest

Relative species abundance is a component of biodiversity and refers to how common or rare a species is relative to other species in a defined location or community (Avolio et al., 2019). Among the five models evaluated for relative species abundance (Null, Lognormal, Mendelbrot, Preemption, and Zipf), three fit in our analysis (Fig. 2). The results of the rank abundance curve showed that yeasts were most abundant in the UFAM area, followed by Piquiá and Carú. If the curve from an area becomes steep, it means that there are few species in that location and the species are common, which is the case of Carú. A shallow slope indicates high evenness as the abundances of different species are similar, as shown in UFAM´s curve, while a steeper curve denotes more species in the area.

The cumulative number of species (collector's curve) also presented UFAM as the area with the most even community since its curve climbed more rapidly than Piquiá and Carú (Fig. 3). The collector's curve of a community gives the expected number of observed species and the order in which samples are added to the total affects the shape of the curve (McCabe, 2011). Variation in curve shape due to accumulation order arises from sampling error, as well as from real heterogeneity among the units sampled. To eliminate this arbitrariness, the sample order may be randomized (Selgrath and Gergel, 2019). With this in mind, we also constructed a rarefaction curve representing the number

of observed species, in which the pooled samples were randomized 1000 times. The rarefaction curve is a plot of the number of species against the number of samples. The species richness was highest in UFAM (Fig. 4).

Beta diversity, which measures the differences between communities, was slightly lower between Piquiá-UFAM than Piquiá-Carú (Fig. 5). The highest dissimilarity was found between UFAM and Carú. UFAM presented the highest beta diversity. We calculated other diversity indices (Shannon, Simpson's Index of Diversity, Simpson's Reciprocal Index, and Pielou's Evenness Index), and the results confirmed UFAM as the most diverse area (Fig. S1).

As previously mentioned, Carú is a floodplain forest (*várzea*), and UFAM and Piquiá are dryland forests (*terra firme*). The first area presents less tree species richness and diversity than the adjacent drylands (Bredin et al., 2020). This pattern was also reported by Ritter et al. (2020) for the fungal community, in which the richness was lower in floodplain areas than in the drylands of the Brazilian Amazonian Forest. It was suggested that floodplain areas are poorer in species than dryland forests because of the impact of the annual flood. Regarding the distinct and important ecosystems of the Amazonian Forest, studies put them in the following order of decreasing plant and animal diversity: dryland tropical forests (*terra firme*); *várzeas*; forests seasonally flooded by unfertile black water rivers (nutrient-poor water, *igapós*); and naturally open areas associated with white-sand soils (*campinas*) (Ritter et al., 2019; Myster, 2016). Fungal communities are highly variable in space and time. Their structures are strongly linked with plants, both in terms of species composition and species richness (Peay et al. 2013). Our findings also showed that the area characterized as *várzea* (Carú) presents a lower

diversity than the dryland forests, but with respect to yeasts that are able to assimilate lignocellulosic sugars. Fungal richness and diversity are positively related to forest tree diversity (Xiao et al., 2019). Considering the studies of Myster (2016) and Ritter et al. (2019), we can hypothesize that the diversity of yeasts associated with rotting wood is higher in dryland forests due to its higher diversity of trees.

UFAM was the area in which we recovered the highest number of candidates for new species (22 candidates), followed by Piquiá (20) and Carú (8). However, more isolates of candidates for new species were recovered in Piquiá (43 isolates) than UFAM (34) and Carú (26). Regarding media, YNB-xylose was the most successful medium for isolating new species (12 candidates) followed by YNB-xylan (10), SBHH (8), SBHH 1:2 (7), SBHH 1:5 (6), CCHH 1:2 (6), CCHH 1:5 (6), and CCHH (2). The number of isolates of candidates for novel species was also highest in YNB-xylose medium (24 isolates). There was not a large difference in the number of candidates for new species recovered in YNB-xylose and xylan compared to SBHH, although we recovered a smaller number of new species from hydrolysates. These results were expected given that hydrolysates are more selective than the other media due to the inhibitors and different sugars in their composition.

Although our results presented a high diversity of yeasts associated with rotting wood in the areas of the Brazilian Amazonian rainforest, this work was carried out using a culture-based method. We also used selective culture media to isolate the yeasts, which is another limitation of our study regarding sampling. This work did not present a portrait of the total diversity in rotting wood because the selective media only allow the growth of specific microorganisms; it was also impossible to access the diversity of non-culturable

species. To capture the total diversity in rotting wood samples, DNA metabarcoding approaches would fit better because they provide the most detailed access to the diversity of microorganisms. The diversity probably would be higher in this study if we had applied metabarcoding technology. Unfortunately, there are no similar studies using this technology to compare with our results. However, we have chosen a culturing method and selective media because our target microorganisms were yeasts capable of growing in lignocellulosic sugars, and we have described the diversity of this small subset, isolated new species, and identified species for future bioprospecting studies.

Phylogenetic placement of possible new species of interest for xylanase production or xylose fermentation

Some possible new species isolated in this work were placed in clades reported to harbor species that can convert xylose into products of great economic interest (Morais et al. 2020). Among the major clades of yeasts associated with rotting wood habitats, five are worth mentioning for their ability to assimilate/ferment lignocellulose-related sugars and/or to produce enzymes that act on this substrate: *Spathaspora*, *Scheffersomyces*, *Spencermartinsiella*, *Sugiyamaella*, and *Cyberlindnera* (Barros et al. 2021, Cadete et al. 2017).

Two possible new species belonging to *Cyberlindnera* were isolated in YNB-xylan and YNB-xylose from samples collected at UFAM. They are closely related to *Cy. japonica* and *Cy. maritima*. BLAST results showed that *Cyberlindnera* sp. 1 differs by three substitutions from *Cy. japonica* in its D1/D2 sequence; in its ITS-5.8S region, it differs from *Candida easanensis* by 27 substitutions and 7 indels, while it differs from *Cy. japonica* by 47 substitutions and 13 indels. *Cyberlindnera* sp. 2 is distinguished from *Cy.*

maritima by nine substitutions in the D1/D2 sequence and 32 substitutions in the ITS region. The phylogenetic tree (Fig. 6) placed this species into a clade that includes *Cy. maritima* and another undescribed species, while *Cyberlindera* sp. 1 was placed in a clade with *Cy. japonica*, *C. easanensis*, *Cy. xylosilytica*, and *Candida maesa.*

Sequence comparison of the ITS-5.8S regions and D1/D2 domains of 24 isolates of *Sugiyamaella* showed that they represent six candidates for novel species (Table 1). The first species was represented by two isolates obtained from rotting wood in YNB-xylan. These isolates presented identical ITS-5.8S and D1/D2 sequences. *Sugiyamaella* sp. 1 UFMG-CM-Y6964 was most closely related to *Su. americana* based on D1/D2 analysis (Fig. 7). The second candidate for a novel species UFMG-CM-Y6968 was represented by twelve isolates, and it was most closely related to *Su. paludigena*. The new *Sugiyamaella* sp. 3, 4, and 5 were closely related to *Su. bahiana*, *Su. bonitensis*, and *Su. trypani*, respectively. Finally, *Sugiyamaella* sp. 6 was related to the described species *Su. americana* and *Su. carassensis*, but it was most closely related to several undescribed *Sugiyamaella* species with sequences deposited in GenBank (Fig. 7). The phylogram in Fig. 7 suggests that the genus *Sugiyamaella* may be polyphyletic because its species are intertwined with representatives of the genera *Trichomonascus*, *Diddensiella*, and *Spencermartinsiella*, although we note that the bootstrap support values are low for many key nodes. Indeed, the families Dipodascaceae and Trichomonascaceae, which contain these genera, are known to not be monophyletic (Shen et al. 2018). The phylogenetic tree also showed two yeasts obtained from rotten wood in this work as representing candidates for novel species in the genus *Spencermartinsiella*. The five isolates of *Spencermartinsiella* sp. 1 differ from *Spen. ligniputridi* and *Spen. silvicola* by 16 and 22

substitutions and seven indels each in D1/D2 domains, respectively, and the phylogram placed it more closely related to *Spen. silvicola*. *Spencermartinsiella* sp. 2 is placed next to the strain *Spencermartinsiella* sp. UFMG-CM-Y3197, which appears to be conspecific, and they are both closely related to *Spen. cellulosicola*.

Currently, the genus *Spathaspora* is composed of a core group that is well supported by phylogenetic analyses of D1/D2 LSU sequences and includes *Sp. passalidarum, Sp. arborariae, Sp. brasiliensis, Sp. suhii, Sp. girioi, Sp. jeffriesii*, *Sp. materiae*, and *Sp. piracicabensis* (Lopes et al. 2016; Cadete and Rosa, 2018). The novel species *Spathaspora* sp. 1 differs by 11 substitutions and one indel in D1/D2 domains from *Sp. arborariae* and 12 substitutions from *Sp. brasiliensis* and *Sp. suhii* (Table 1). Analysis of D1/D2 domain sequences suggests that this yeast represents a candidate for a novel species of the clade *Spathaspora* that is phylogenetically related to *Sp. arborariae* (Fig. 8). Sequences of both the ITS-5.8S and D1/D2 domains from both isolates of *Spathaspora* sp. 1 were identical.

Unlike *Spathaspora* sp. 1, we obtained only one isolate of *Spathaspora* sp. 2. It was placed phylogenetically outside of the core group, sister to *Sp. boniae*, in a clade that also contains *C. albicans* and *C. dubliniensis* (Fig. 8). Morais et al. (2017), based on the analysis of 510 conserved genes shared by the strain *Sp. boniae* UFMG-CM-Y306^{T} and 34 other species of the CUG Ser-1 clade, placed *Sp. boniae* outside of the clade containing known *Spathaspora* with available genome sequences. Instead, these analyses placed it as an outgroup of the *C. albicans*/*Lodderomyces* clade.

Although *Scheffersomyces* species have remarkable abilities to ferment xylose to ethanol or xylitol, we do not have enough data for phylogenetic analysis because the ITS

region and D1/D2 domain sequences are known to be insufficient to distinguish the new species in this genus. A multilocus dataset, including sequencing of *XYL* and *RPB1* genes, would be necessary for a well-supported phylogeny (Urbina and Blackwell, 2012). The purpose of the trees shown here is primarily to demonstrate the potential novelty of the species isolated in this work and point to their nearest relatives. Formal description and classification of the new species may arise from future studies using genome-based analyses of all relevant species.

Conclusions

This is the first study in the Brazilian Amazonian rainforest to use minimal and complex media to isolate yeasts from rotting wood in floodplain and dryland areas. The results revealed a high diversity of yeast species associated with that substrate in dryland forest, including many candidates for novel species, which represented 36% of all identified species. The strategy of using different types of media to isolate yeast species can be used to explore the diversity of yeasts from several substrates in different biomes. Also, the use of hemicellulosic hydrolysates favors the isolation of yeast species capable of growing in the presence of inhibitors. These yeasts could have the potential to be used directly or to be a source of genes in experiments to genetically improve strain performance for the conversion of lignocellulosic sugars into biofuels and bioproducts, such as ethanol and xylitol.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

KOB, GFLS, MAA collected the rotting wood samples.
KOB, FBMA, GM isolated and identified species.
KOB performed the diversity and phylogenetic analyses and wrote the manuscript.
FP, SSS, RCLB provided the hemicellulosic hydrolysates.
CTH, TKS, and CAR edited the manuscript, and all co-authors approved it.
KOB, TKS, CTH, and CAR provided mentorship at various stages of the study.

References

Ali, S. S., Wu, J., Xie, R., Zhou, F., Sun, J., and Huang, M. (2017). Screening and characterizing of xylanolytic and xylose-fermenting yeasts isolated from the wood-feeding termite, Reticulitermes chinensis. *PLoS One*, 12(7), e0181141.

Alvarez-Perez, S., Mateos, A., Dominguez, L., Martinez-Nevado, E., Rodriguez-Bertos, A., Blanco, J. L., and Garcia, M. E. (2012). First isolation of the anamorph of *Kazachstania* heterogenica from a fatal infection in a primate host. *Sabouraudia*, 50(2), 193-196.

Alves, R. C., Tres, A., Soares, R. V., Wendling, W. T., and Tetto, A. F. (2019). Classificação climática para o estado do Amazonas segundo as zonas de vida de Holdridge. *Revista de Ciências Agrárias Amazonian Journal of Agricultural and Environmental Sciences*, 62.

Antonelli, A., Zizka, A., Carvalho, F. A., Scharn, R., Bacon, C. D., Silvestro, D., and Condamine, F. L. (2018). Amazonia is the primary source of Neotropical biodiversity. *PNAS*, 115(23), 6034-6039.

Araujo, F. V., Rosa, C. A., Freitas, L. F., Lachance, M. A., Vaughan-Martini, A., Mendonça-Hagler, L. C., & Hagler, A. N. (2012). *Kazachstania bromeliacearum* sp. nov., a yeast species from water tanks of bromeliads. *International Journal of Systematic and Evolutionary Microbiology*, 62(Pt_4), 1002-1006.

Avolio, M. L., Carroll, I. T., Collins, S. L., Houseman, G. R., Hallett, L. M., Isbell, F.,... and Wilcox, K. R. (2019). A comprehensive approach to analyzing community dynamics using rank abundance curves. *Ecosphere*, 10(10), e02881.

Barros, K. O., Souza, R. M., Palladino, F., Cadete, R. M., Santos, A. R. O., Goes-Neto, A., Berkov, A., Zilli, J.E., Vital, M.J.S, Lachance, M.A. and Rosa, C. A. (2021). *Cyberlindnera dasilvae* sp. nov., a xylitol-producing yeast species isolated from rotting wood and frass of cerambycid larva. *International Journal of Systematic and Evolutionary Microbiology*, 71(9), 004986.

Barros KO, Garcia-Acero AM, and Rosa CA. (2020). Non-conventional yeasts with potential for production of second-generation ethanol. In: Carey, G. R. (editor). Biofuels: Advances in Research and Applications. Nova Science Publishers, New York. pp. 109-150.

Bellutti, N., Gallmetzer, A., Innerebner, G., Schmidt, S., Zelger, R., and Koschier, E. H. (2018). Dietary yeast affects preference and performance in *Drosophila suzukii*. *Journal of Pest Science*, 91(2), 651-660.

Bensasson, D., Dicks, J., Ludwig, J. M., Bond, C. J., Elliston, A., Roberts, I. N., and James, S. A. (2019). Diverse lineages of *Candida albicans* live on old oaks. *Genetics*, 211(1), 277-288.

Bhardwaj, N., Kumar, B., and Verma, P. (2019). A detailed overview of xylanases: an emerging biomolecule for current and future prospective. *Bioresources and Bioprocessing*, 6(1), 1-36.

Blackwell, M. (2017). Made for each other: ascomycete yeasts and insects. *Microbiology Spectrum*, 5(3), 5-3.

Bonatto, C., Venturin, B., Mayer, D. A., Bazoti, S. F., de Oliveira, D., Alves Jr, S. L., and Treichel, H. (2020). Experimental data and modelling of 2G ethanol production by *Wickerhamomyces* sp. UFFS-CE-3.1. 2. *Renewable Energy*, 145, 2445-2450.

Boontham, W., Limtong, S., Rosa, C. A., Lopes, M. R., Vital, M. J., and Srisuk, N. (2017). *Cyberlindnera tropicalis* fa, sp. nov., a novel yeast isolated from tropical regions. *International Journal of Systematic and Evolutionary Microbiology*, 67(8), 2569-2573.

Bredin, Y. K., Hawes, J. E., Peres, C. A., and Haugaasen, T. (2020). Structure and composition of Terra Firme and seasonally flooded Várzea forests in the Western Brazilian Amazon. *Forests*, 11(12), 1361.

Cadete, R. M., and Rosa, C. A. (2018). The yeasts of the genus *Spathaspora*: potential candidates for second-generation biofuel production. *Yeast*, 35(2), 191-199.

Cadete, R. M., Lopes, M. R., and Rosa, C. A. (2017). Yeasts associated with decomposing plant material and rotting wood. In: Buzzini P, Lachance MA, Yurkov A. (editors). *Yeasts in Natural Ecosystems: diversity*. Springer, Cham. pp. 265-292.

Cadete, R. M., Melo-Cheab, M. A., Viana, A. L., Oliveira, E. S., Fonseca, C., and Rosa, C. A. (2016a). The yeast *Scheffersomyces amazonensis* is an efficient xylitol producer. *World Journal of Microbiology and Biotechnology*, 32(12), 1-5.

Cadete, R. M., de Las Heras, A. M., Sandström, A. G., Ferreira, C., Gírio, F., Gorwa-Grauslund, M. F.,... and Fonseca, C. (2016b). Exploring xylose metabolism in *Spathaspora* species: *XYL1.2* from *Spathaspora passalidarum* as the key for efficient anaerobic xylose fermentation in metabolic engineered *Saccharomyces cerevisiae*. *Biotechnology for Biofuels*, 9(1), 1-14.

Cadete, R. M., Cheab, M. A., Santos, R. O., Safar, S. V., Zilli, J. E., Vital, M. J.,... and Rosa, C. A. (2015). *Cyberlindnera xylosilytica* sp. nov., a xylitol-producing yeast species isolated from lignocellulosic materials. *International Journal of Systematic and Evolutionary Microbiology*, 65(Pt_9), 2968-2974.

Cadete, R. M., Melo, M. A., Dussan, K. J., Rodrigues, R. C., Silva, S. S., Zilli, J. E.,... and Rosa, C. A. (2012). Diversity and physiological characterization of D-xylose-fermenting yeasts isolated from the Brazilian Amazonian Forest. *PLoS One*, 7, e43135

Cadete, R. M., Melo, M. A., Lopes, M. R., Pereira, G. M., Zilli, J. E., Vital, M. J.,... and Rosa, C. A. (2012). *Candida amazonensis* sp. nov., an ascomycetous yeast isolated from rotting wood in the Amazonian forest. *International Journal of Systematic and Evolutionary Microbiology*, 62(Pt_6), 1438-1440.

Carvalho, J. K., Panatta, A. A. S., Silveira, M. A. D., Tav, C., Johann, S., Rodrigues, M. L. F., and Martins, C. V. B. (2021). Yeasts isolated from a lotic continental environment in Brazil show potential to produce amylase, cellulase and protease. *Biotechnology Reports*, 30, e00630.

Chai, C. Y., Gao, W. L., Yan, Z. L., and Hui, F. L. (2022). Four new species of Trichomonascaceae (Saccharomycetales, Saccharomycetes) from Central China. *MycoKeys*, *90*, 1-18.

Cheng, Y. T., and Yang, C. F. (2016). Using strain *Rhodotorula mucilaginosa* to produce carotenoids using food wastes. *Journal of Taiwan Institute of Chemistry and. Engineers*, 61, 270-275.

da Costa Neto, D. J. C., and Morais, P. B. (2020). The vectoring of *Starmerella* species and other yeasts by stingless bees in a Neotropical savanna. *Fungal Ecology*, 47, 100973.

Daniel, H. M., Lachance, M. A., and Kurtzman, C. P. (2014). On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. *Antonie van Leeuwenhoek*, 106(1), 67-84.

Geijer, C., Faria-Oliveira, F., Moreno, A. D., Stenberg, S., Mazurkewich, S., and Olsson, L. (2020). Genomic and transcriptomic analysis of *Candida intermedia* reveals the genetic determinants for its xylose-converting capacity. *Biotechnology for Biofuels*, 13(1), 1-15.

Gomes, F. C., Safar, S. V., Marques, A. R., Medeiros, A. O., Santos, A. R. O., Carvalho, C.,... and Rosa, C. A. (2015). The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of *Vriesea minarum*, an endangered bromeliad species in Brazil, and the description of *Occultifur brasiliensis* fa, sp. nov. *Antonie van Leeuwenhoek*, 107(2), 597-611.

Goswami, M., Bhattacharyya, P., Mukherjee, I., and Tribedi, P. (2017). Functional diversity: an important measure of ecosystem functioning. *Advances in Microbiology*, 7(01), 82.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3), 307-321.

Guzmán, B., Lachance, M. A., and Herrera, C. M. (2013). Phylogenetic analysis of the angiosperm-floricolous insect-yeast association: Have yeast and angiosperm lineages co-diversified?. *Molecular Phylogenetics and Evolution*, 68(2), 161-175.

Hamby, K. A., and Becher, P. G. (2016). Current knowledge of interactions between *Drosophila* suzukii and microbes, and their potential utility for pest management. *Journal of Pest Science*, 89(3), 621-630.

Hamidi, M., Gholipour, A. R., Delattre, C., Sesdighi, F., Seveiri, R. M., Pasdaran, A., Kheirandish, S., Pierre, G., Kozani, P.S., Kozani, P. and Karimitabar, F. (2020). Production, characterization and biological activities of exopolysaccharides from a new cold-adapted yeast: *Rhodotorula mucilaginosa* sp. GUMS16. *International Journal of Biological Macromolecules*, 151, 268-277.

Han, S. M., Kim, J. Y., & Lee, J. S. (2019). Isolation of wild yeasts from the water and riverside soil of Geumgang midstream in Sejong city, Korea, and characterization of unrecorded wild yeasts. *The Korean Journal of Mycology*, 47(1), 51-61.

Hittinger, C. T. (2013). *Saccharomyces* diversity and evolution: a budding model genus. *Trends in Genetics*, 29(5), 309-317.

Hui, F. L., Chen, L., Li, Z. H., Niu, Q. H., and Ke, T. (2013). *Metschnikowia henanensis* sp. nov., a new anamorphic yeast species isolated from rotten wood in China. *Antonie van Leeuwenhoek*, 103(4), 899-904.

Ioannou, P., Vamvoukaki, R., and Samonis, G. (2019). *Rhodotorula* species infections in humans: A systematic review. *Mycoses*, 62(2), 90-100.

Jacques, N., Sarilar, V., Urien, C., Lopes, M. R., Morais, C. G., Uetanabaro, A. P. T.,... and Casaregola, S. (2016). Three novel ascomycetous yeast species of the *Kazachstania* clade, *Kazachstania saulgeensis* sp. nov., *Kazachstania serrabonitensis* sp. nov. and *Kazachstania australis* sp. nov. Reassignment of *Candida humilis* to *Kazachstania humilis* fa comb. nov. and *Candida pseudohumilis* to *Kazachstania pseudohumilis* fa comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 66(12), 5192-5200.

Jeffries, T. W., and Jin, Y. S. (2000). Ethanol and thermotolerance in the bioconversion of xylose by yeasts. *Advances in Applied Microbiology*, 48, 221-268.

Kim, B. R., Shin, J., Guevarra, R. B., Lee, J. H., Kim, D. W., Seol, K. H.,... and Isaacson, R. E. (2017). Deciphering diversity indices for a better understanding of microbial communities. *Journal of Microbiology and Biotechnology*, 27(12), 2089-2093.

Kumar, A., and Ram, C. (2021). Agave biomass: A potential resource for production of value-added Products. *Environmental Sustainability*, 4(2), 245-259.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874.

Kurtzman CP, Boekhout T. (2017). Yeasts as distinct life forms of fungi. In Yeasts in natural ecosystems: ecology (pp. 1-37). Springer, Cham.

Kurtzman, CP. *Scheffersomyces* Kurtzman & M. Suzuki (2010). In: Kurtzman CP, Fell JW, Boekhout T (editors). *The Yeasts, a Taxonomic Study*, 5th ed, vol. 1. Amsterdam: Elsevier; 2011. pp. 773-777.

Kurtzman, C. P., and 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(4), 331-371.

Lachance MA, Boekhout T, Scorzetti T, Fell JW, Kurtzman CP. *Candida* Berkhout (1923). In: Kurtzman CP, Fell JW, Boekhout T (editors). *The Yeasts, a Taxonomic Study*, 5th ed, vol. 1. Amsterdam: Elsevier; 2011. pp. 987-1278.

Lachance, M. A., Bowles, J. M., Starmer, W. T., and Barker, J. S. F. (1999). *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. *Canadian Journal of Microbiology*, 45(2), 172-177.

Lara, C. A., Santos, R. O., Cadete, R. M., Ferreira, C., Marques, S., Girio, F.,... and Fonseca, C. (2014). Identification and characterisation of xylanolytic yeasts isolated from decaying wood and sugarcane bagasse in Brazil. *Antonie van Leeuwenhoek*, 105(6), 1107-1119.

Lee, C. F., Yao, C. H., Liu, Y. R., Young, S. S., and Chang, K. S. (2009). *Kazachstania wufongensis* sp. nov., an ascosporogenous yeast isolated from soil in Taiwan. *Antonie van Leeuwenhoek*, 95(4), 335-341.

Lewis, M. T., and Hamby, K. A. (2019). Differential impacts of yeasts on feeding behavior and development in larval *Drosophila suzukii* (Diptera: Drosophilidae). *Scientific Reports*, 9(1), 1-12.

Libkind, D., Brizzio, S., and Van Broock, M. (2004). *Rhodotorula mucilaginosa*, a carotenoid producing yeast strain from a Patagonian high-altitude lake. *Folia Microbiologica*, 49(1), 19-25.

Libkind, D., Brizzio, S., Ruffini, A., Gadanho, M., van Broock, M., and Paulo Sampaio, J. (2003). Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. *Antonie van Leeuwenhoek*, 84(4), 313-322.

Limtong, S., Yongmanitchai, W., Kawasaki, H., and Fujiyama, K. (2009). *Wickerhamomyces edaphicus* sp. nov. and *Pichia jaroonii* sp. nov., two ascomycetous yeast species isolated from forest soil in Thailand. *FEMS Yeast Research*, 9(3), 504-510.

Liu, Y., Cruz-Morales, P., Zargar, A., Belcher, M. S., Pang, B., Englund, E.,... and Keasling, J. D. (2021). Biofuels for a sustainable future. *Cell*, 184(6), 1636-1647.

Lopes, M. R., Lara, C. A., Moura, M. E., Uetanabaro, A. P. T., Morais, P. B., Vital, M. J., and Rosa, C. A. (2018a). Characterisation of the diversity and physiology of cellobiose-fermenting yeasts isolated from rotting wood in Brazilian ecosystems. *Fungal Biology*, 122(7), 668-676..

Lopes, M. R., Batista, T. M., Franco, G. R., Ribeiro, L. R., Santos, A. R., Furtado, C.,... and Rosa, C. A. (2018b). *Scheffersomyces stambukii* fa, sp. nov., a D-xylose-fermenting species isolated from rotting wood. *International Journal of Systematic and Evolutionary Microbiology*, 68(7), 2306-2312.

Lopes, M. R., Morais, C. G., Kominek, J., Cadete, R. M., Soares, M. A., Uetanabaro, A. P. T.,... and Rosa, C. A. (2016). Genomic analysis and D-xylose fermentation of three

novel *Spathaspora* species: *Spathaspora girioi* sp. nov., *Spathaspora hagerdaliae* fa, sp. nov. and *Spathaspora gorwiae* fa, sp. nov. *FEMS Yeast Research*, 16(4), fow044.

McCabe, D. J. (2011). Sampling biological communities. *Nature Education Knowledge 2* (11), 13(2).

Mankowski, M. E., Morrell, J. J., and Lebow, P. K. (2021). Effects on brood development in the carpenter ant *Camponotus vicinus* Mayr after exposure to the yeast associate *Schwanniomyces polymorphus* Kloecker. *Insects*, 12(6), 520.

Martínez, Á. T., Speranza, M., Ruiz-Dueñas, F. J., Ferreira, P., Camarero, S., Guillén, F.,... and Río Andrade, J. C. D. (2005). Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology*, 8, 195–204

Myster, R. W. (2016). The physical structure of forests in the Amazon Basin: a review. *The Botanical Review*, 82(4), 407-427.

Morais, C. G., Sena, L. M., Lopes, M. R., Santos, A. R. O., Barros, K. O., Alves, C. R.,... and Rosa, C. A. (2020). Production of ethanol and xylanolytic enzymes by yeasts inhabiting rotting wood isolated in sugarcane bagasse hydrolysate. *Fungal Biology*, 124(7), 639-647.

Morais, C. G., Batista, T. M., Kominek, J., Borelli, B. M., Furtado, C., Moreira, R. G.,... and Rosa, C. A. (2017). *Spathaspora boniae* sp. nov., a D-xylose-fermenting species in the *Candida albicans/Lodderomyces* clade. *International Journal of Systematic and Evolutionary Microbiology*, 67(10), 3798-3805.

Moreira, L. R. S. (2016). Insights into the mechanism of enzymatic hydrolysis of xylan. *Applied Microbiology and Biotechnology*, 100(12), 5205-5214.

Morais, C. G., Cadete, R. M., Uetanabaro, A. P. T., Rosa, L. H., Lachance, M. A., and Rosa, C. A. (2013). D-xylose-fermenting and xylanase-producing yeast species from rotting wood of two Atlantic Rainforest habitats in Brazil. *Fungal Genetics and Biology*, 60, 19-28.

Nakanishi, S. C., Soares, L. B., Biazi, L. E., Nascimento, V. M., Costa, A. C., Rocha, G. J. M., and Ienczak, J. L. (2017). Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by *Spathaspora passalidarum* and *Scheffersomyces stipitis*. *Biotechnology and Bioengineering*, 114(10), 2211-2221.

Narisetty, V., Castro, E., Durgapal, S., Coulon, F., Jacob, S., Kumar, D.,... and Kumar, V. (2021). High level xylitol production by *Pichia fermentans* using non-detoxified xylose-rich sugarcane bagasse and olive pits hydrolysates. *Bioresource Technology*, 342, 126005.

Nguyen, N. H., Suh, S. O., Marshall, C. J., and Blackwell, M. (2006). Morphological and ecological similarities: wood-boring beetles associated with novel xylose-fermenting yeasts, *Spathaspora passalidarum* gen. sp. nov. and *Candida jeffriesii* sp. nov. *Mycological Research*, 110(10), 1232-1241.

O'Donnell K. Fusarium and its near relatives. In: Reynolds DR, 604 Taylor JW (editors), The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematic. CAB International, Oregon; 1993. pp. 225-233.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solym P, Stevens H, Szoecs E, Wagner E. *vegan: Community Ecology Package*, 2020.

Opulente, D. A., Langdon, Q. K., Buh, K. V., Haase, M. A., Sylvester, K., Moriarty, R. V.,... and Hittinger, C. T. (2019). Pathogenic budding yeasts isolated outside of clinical settings. *FEMS Yeast Research*, 19(3), foz032.

Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35(3), 526-528.

Parolin, P., Ferreira, L. V., Albernaz, A. L., and Almeida, S. S. (2004). Tree species distribution in Várzea forests of Brazilian Amazonia. *Folia Geobotanica*, 39(4), 371-383.

Peay, K. G., Baraloto, C., and Fine, P. V. (2013). Strong coupling of plant and fungal community structure across western Amazonian rainforests. *The ISME Journal*, 7(9), 1852-1861.

Péter G, Takashima M, Čadež N. Yeast habitats: different but global (2017). In: Buzzini P, Lachance MA, Yurkov A. (editors). Yeasts in Natural Ecosystems: diversity. Springer, Cham. pp. 39-71.

Ricotta, C., and Podani, J. (2017). On some properties of the Bray-Curtis dissimilarity and their ecological meaning. *Ecological Complexity*, 31, 201-205.

Ritter, C. D., Dunthorn, M., Anslan, S., de Lima, V. X., Tedersoo, L., Nilsson, R. H., and Antonelli, A. (2020). Advancing biodiversity assessments with environmental DNA: Long-read technologies help reveal the drivers of Amazonian fungal diversity. *Ecology and Evolution*, 10(14), 7509-7524.

Ritter, C. D., Zizka, A., Barnes, C., Nilsson, R. H., Roger, F., and Antonelli, A. (2019). Locality or habitat? Exploring predictors of biodiversity in Amazonia. *Ecography*, 42(2), 321-333.

Roche, M. D., Pearse, I. S., Bialic-Murphy, L., Kivlin, S. N., Sofaer, H. R., and Kalisz, S. (2021). Negative effects of an allelopathic invader on AM fungal plant species drive community level responses. *Ecology*, 102(1), e03201.

Rodrigues RCLB, Rocha, G. J., Rodrigues Jr, D., Helcio Filho, J. I., Maria das Graças, A. F., and Pessoa Jr, A. (2010). Scale-up of diluted sulfuric acid hydrolysis for producing sugarcane bagasse hemicellulosic hydrolysate (SBHH). *Bioresource Technology,* 101(4), 1247-1253.

Rönnander, J., and Wright, S. A. I. (2021). Growth of wood-inhabiting yeasts of the Faroe Islands in the presence of spent sulphite liquor. *Antonie van Leeuwenhoek*, 114(6), 649-666.

Sampaio JP. *Rhodotorula* Harrison (1928). In: Kurtzman CP, Fell JW, Boekhout T (editors). *The Yeasts, a Taxonomic Study*, 5th ed, vol. 1. Amsterdam: Elsevier; 2011. pp. 1873-1927.

Sampaio, J. P., Gadanho, M., Santos, S., Duarte, F. L., Pais, C., Fonseca, A., & Fell, J. W. (2001). Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium: Rhodosporidium kratochvilovae* and related anamorphic species. *International Journal of Systematic and Evolutionary Microbiology*, 51(2), 687-697.

Santos, A. R. D. O., Lee, D. K., Ferreira, A. G., do Carmo, M. C., Rondelli, V. M., Barros, K. O. Rosa, C.A, and Lachance, M. A. (2020). The yeast community of *Conotelus* sp. (Coleoptera: Nitidulidae) in Brazilian passionfruit flowers (*Passiflora edulis*) and description of *Metschnikowia amazonensis* sp. nov., a large spored clade yeast. *Yeast*, 37(3), 253-260.

Sato, T. K., Tremaine, M., Parreiras, L. S., Hebert, A. S., Myers, K. S., Higbee, A. J.,... and Landick, R. (2016). Directed evolution reveals unexpected epistatic interactions that alter metabolic regulation and enable anaerobic xylose use by *Saccharomyces cerevisiae*. *PLoS Genetics*, *12*(10), e1006372.

Sayers, E. W., Beck, J., Bolton, E. E., Bourexis, D., Brister, J. R., Canese, K., Comeau, D. C., Funk, K., Kim, S., Klimke, W., Marchler-Bauer, A. (2021). Database resources of the national center for biotechnology information. *Nucleic Acids Research*, 49(D1), p. D10.

Seibold, S., Müller, J., Baldrian, P., Cadotte, M. W., Štursová, M., Biedermann, P. H.,... and Bässler, C. (2019). Fungi associated with beetles dispersing from dead wood–Let's take the beetle bus!. *Fungal Ecology*, 39, 100-108.

Selgrath, J. C., and Gergel, S. E. (2019). How Much is Enough? Improving Participatory Mapping Using Area Rarefaction Curves. *Land*, 8(11), 166.

Sena, L. M., Morais, C. G., Lopes, M. R., Santos, R. O., Uetanabaro, A., Morais, P. B.,... and Rosa, C. A. (2017). d-Xylose fermentation, xylitol production and xylanase activities by seven new species of *Sugiyamaella*. *Antonie van Leeuwenhoek*, 110(1), 53-67.

Shen XX, Opulente DA, Kominek J, Zhou X, Steenwyk JL, Buh KV… Hittinger CT, and Rokas A. (2018). Tempo and mode of genome evolution in the budding yeast subphylum. *Cell*. 175(6), 1533-1545.

Shi, C. F., Zhang, K. H., Chai, C. Y., Yan, Z. L., and Hui, F. L. (2021). Diversity of the genus *Sugiyamaella* and description of two new species from rotting wood in China. *MycoKeys*, 77, 27.

Souza, G. D. F. L.., Luana, T. C. N. V., Samila, R. P. N., and Maxwel, A. A. (2017). Efficient production of second-generation ethanol and xylitol by yeasts from Amazonian beetles (*Coleoptera*) and their galleries. *African Journal of Microbiology Research*, 11(20), 814-824.

Spurley, W. J., Fisher, K. J., Langdon, Q. K., Buh, K. V., Jarzyna, M., Haase, M. A.,... and Hittinger, C. T. (2022). Substrate, temperature, and geographical patterns among nearly 2000 natural yeast isolates. *Yeast*, 39(1-2), 55-68.

Stefanini, I. (2018). Yeast-insect associations: It takes guts. *Yeast*, 35(4), 315-330.

Suh, S. O., and Zhou, J. J. (2011). *Kazachstania intestinalis* sp. nov., an ascosporogenous yeast from the gut of passalid beetle *Odontotaenius disjunctus*. *Antonie van Leeuwenhoek*, 100(1), 109-115.

Suh, S. O., Marshall, C. J., Mchugh, J. V., and Blackwell, M. (2003). Wood ingestion by passalid beetles in the presence of xylose fermenting gut yeasts. *Molecular Ecology*, 12(11), 3137-3145.

Sun, J., Xia, Y., and Ming, D. (2020). Whole-genome sequencing and bioinformatics analysis of *Apiotrichum mycotoxinivorans*: predicting putative zearalenone-degradation enzymes. *Frontiers in Microbiology,* 11, 1866.

Suzuki M, Kurtzman, CP. *Schwanniomyces Klöcker* emend. M. Suzuki & Kurtzman (2010). In: Kurtzman CP, Fell JW, Boekhout T (editors). *The Yeasts, a Taxonomic Study*, 5th ed, vol. 1. Amsterdam: Elsevier; 2011. pp. 785-794.

Sylvester, K., Wang, Q. M., James, B., Mendez, R., Hulfachor, A. B., & Hittinger, C. T. (2015). Temperature and host preferences drive the diversification of *Saccharomyces* and other yeasts: a survey and the discovery of eight new yeast species. *FEMS Yeast Research*, 15(3), fov002.

Urbina, H., Frank, R., and Blackwell, M. (2013). *Scheffersomyces cryptocercus*: a new xylose-fermenting yeast associated with the gut of wood roaches and new combinations in the *Sugiyamaella* yeast clade. *Mycologia*, 105(3), 650-660.

Urbina, H., and Blackwell, M. (2012). Multilocus phylogenetic study of the Scheffersomyces yeast clade and characterization of the N-terminal region of xylose reductase gene. *PLoS One*, 7(6), e39128.

Urien, C., Legrand, J., Montalent, P., Casaregola, S., and Sicard, D. (2019). Fungal species diversity in French bread sourdoughs made of organic wheat flour. *Frontiers in Microbiology*, 10, 201.

Vaz, A. B., Fonseca, P. L., Leite, L. R., Badotti, F., SALIM, A. C., Araujo, F. M.,... and Goes-Neto, A. (2017). Using next-generation sequencing (NGS) to uncover diversity of wood-decaying fungi in neotropical Atlantic forests. *Phytotaxa*, 295(1), 1-21.

White TJ, Bruns T, Lee SJWT, Taylor JW. Amplification and 660 direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (editors), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego; 1990. pp.315-322.

Xiao, W., Zhao, J., Yan, X., and Guan, Q. (2019). Tree Diversity Determines the Diversity of the Taxonomic and Functional Structure of the Fungal Community in Forest Litter in Southern China. *Forest Science*, 65(1), 40-47.

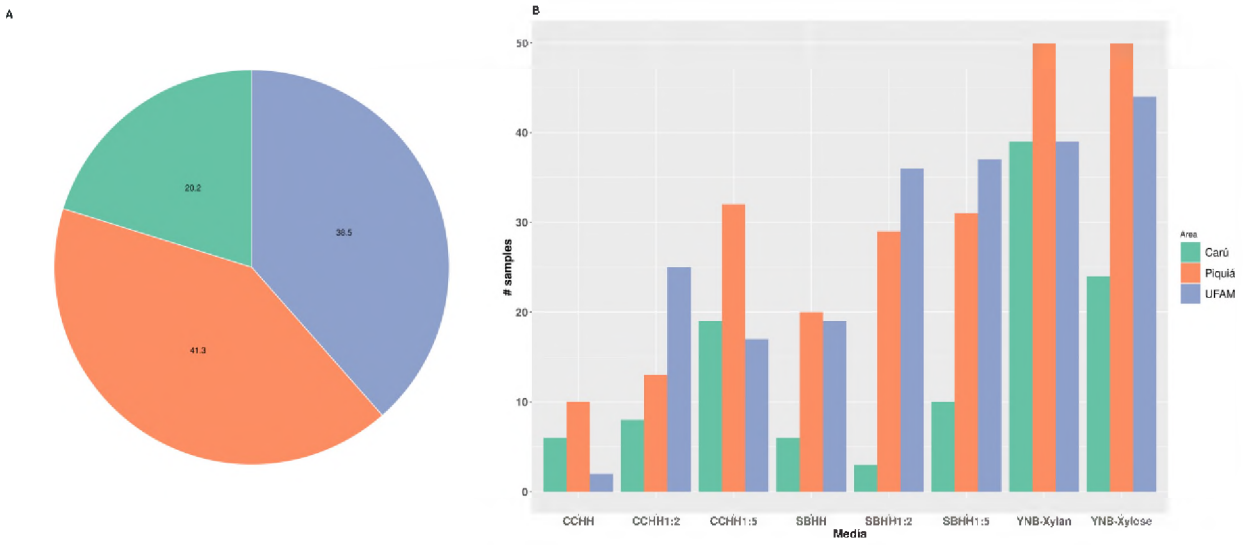

Figure 1. a) Percentage of samples isolated by area; b) distribution of isolates by medium and area. YNB – Yeast Nitrogen Base, SBHH – Sugarcane bagasse hemicellulosic hydrolysate, SBHH - Sugarcane bagasse hemicellulosic hydrolysate diluted 1:2; SBHH 1:5 - Sugarcane bagasse hemicellulosic hydrolysate diluted 1:5; CCHH – corncob hemicellulosic hydrolysate; CCHH 1:2 - corncob hemicellulosic hydrolysate diluted 1:2; CCHH 1:5 - corncob hemicellulosic hydrolysate diluted 1:5.

Table 1. Identification of candidates for novel yeast species isolated in the Amazonian Rainforest sites based on the sequences of the ITS region and D1/D2 domains of the large subunit of the rRNA gene.

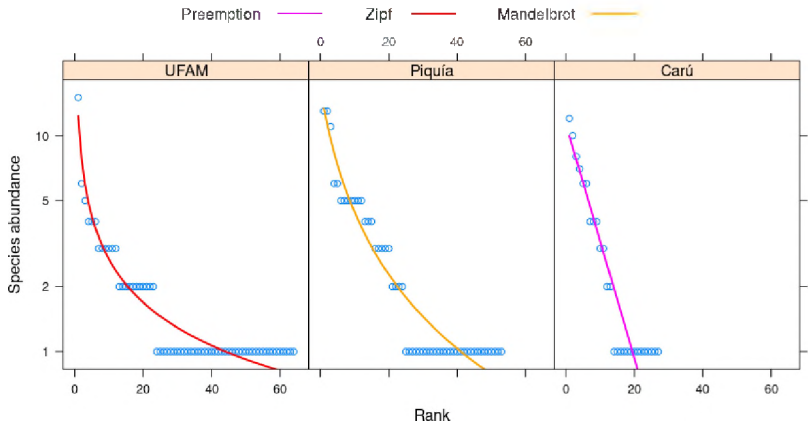

Figure 2. Rank abundance distribution per collecting site (UFAM, Piquiá, and Carú). The Y axis is in log scale and represents the relative abundance. The X axis is the abundance rank, which means that the most abundant species is in rank 1, and the second most abundant is 2. Each blue circle is one species with its respective number of observations. A steep gradient indicates low evenness, and a shallow curve means high evenness. The higher the curve, the more yeast species were found in the area. The Carú site had the fewest species and lowest evenness, while UFAM showed the highest evenness and most species. Five models were evaluated for relative species abundance (Null, Lognormal, Mendelbrot, Preemption, and Zipf), and three fit in our analysis as shown on the plots.

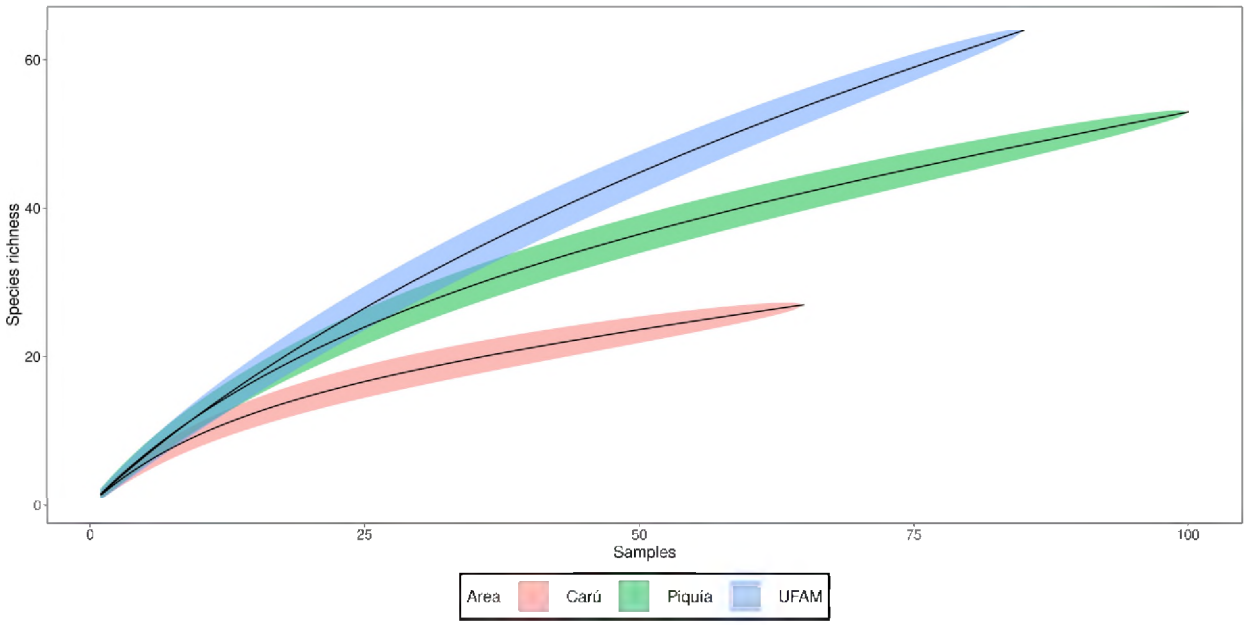

Figure 3. Cumulative number of yeast species recovered in the collecting sites. Areas below and above the curves are the standard deviations.

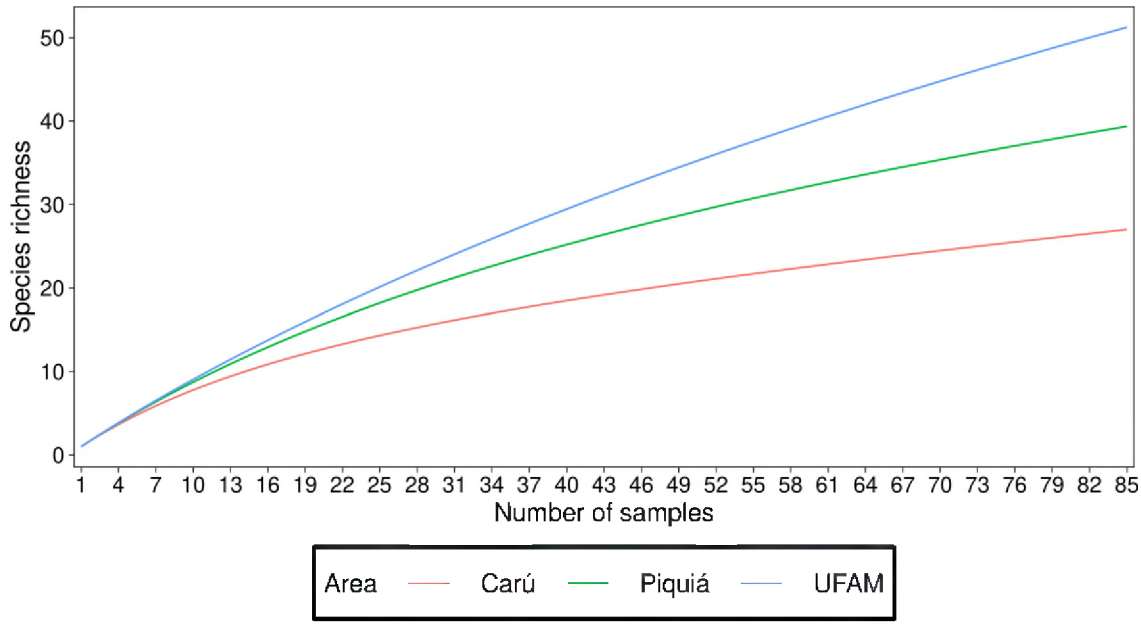

Figure 4. Rarefaction curve of the observed number of yeast species per location.

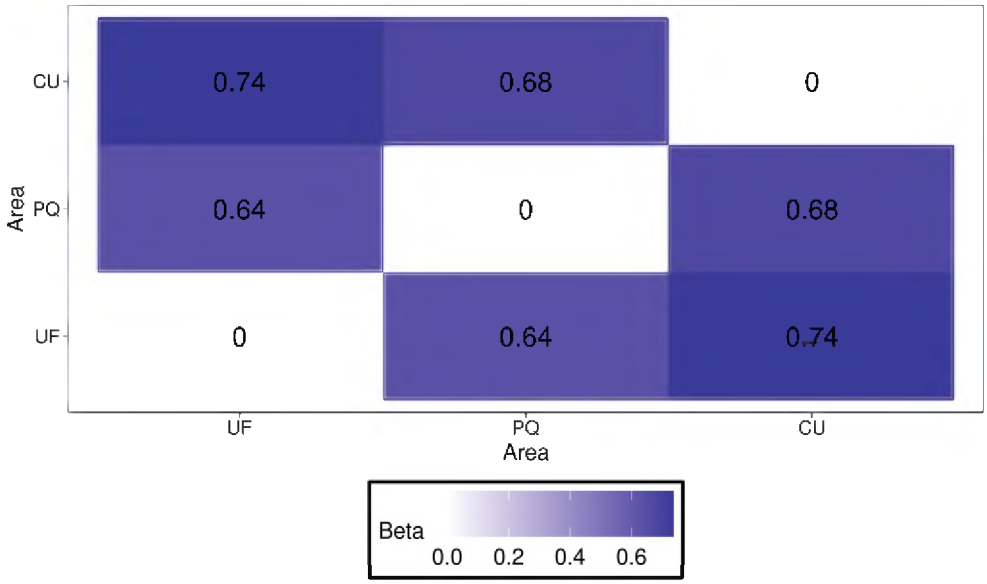

Figure 5. Beta diversity of the locations using a normalized scale from 0 to 1. The higher the value, the more distinct the yeast diversity between the areas. Abbreviations: UF, UFAM; PQ, Piquiá; CU, Carú.

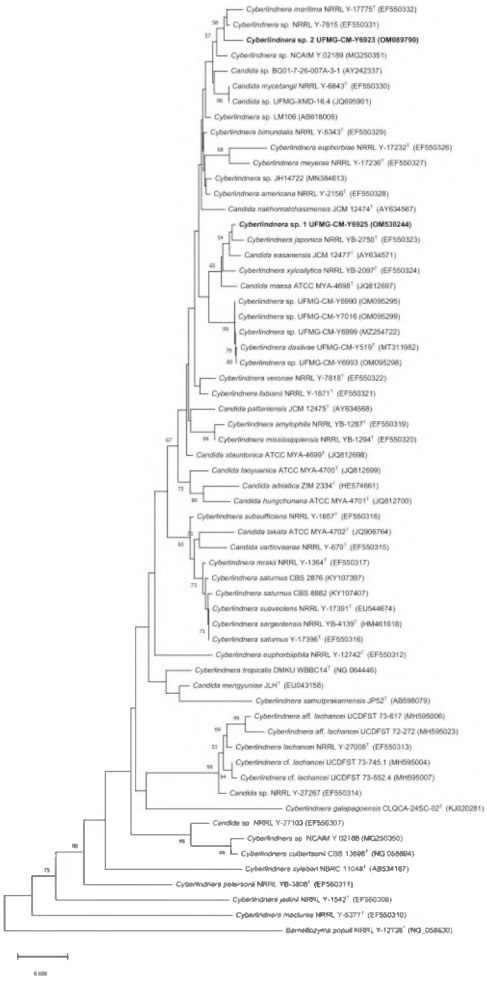

Figure 6. Neighbor-joining phylogenetic tree showing the placement of candidates for new species among members of the *Cyberlindnera* clade based on the D1/D2 domains of the LSU rRNA gene sequences. Numbers at the nodes represent bootstrap values based on 1000 pseudoreplicates. Bar, 0.02 substitutions per nucleotide position.

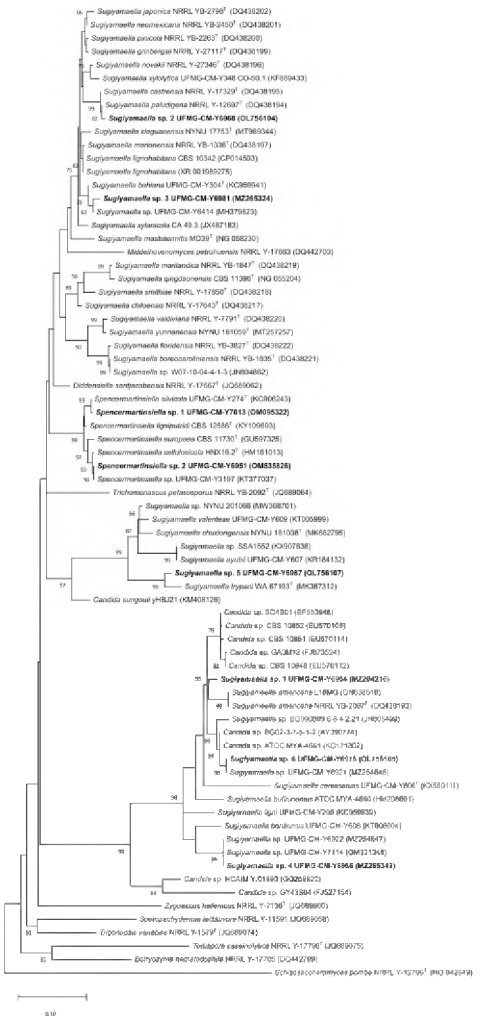

Figure 7. Maximum-likelihood phylogram based on sequences of the D1/D2 domains of the

LSU rRNA gene sequences, which show the placements of the candidates for new species of *Sugiyamaella* and *Spencermartinsiella*. The consistency of the phylogenetic signal was evaluated by bootstrapping from 1000 pseudoreplicates. A total of 433 positions were retained in the final dataset. Bar, 0.10 substitutions per nucleotide position.

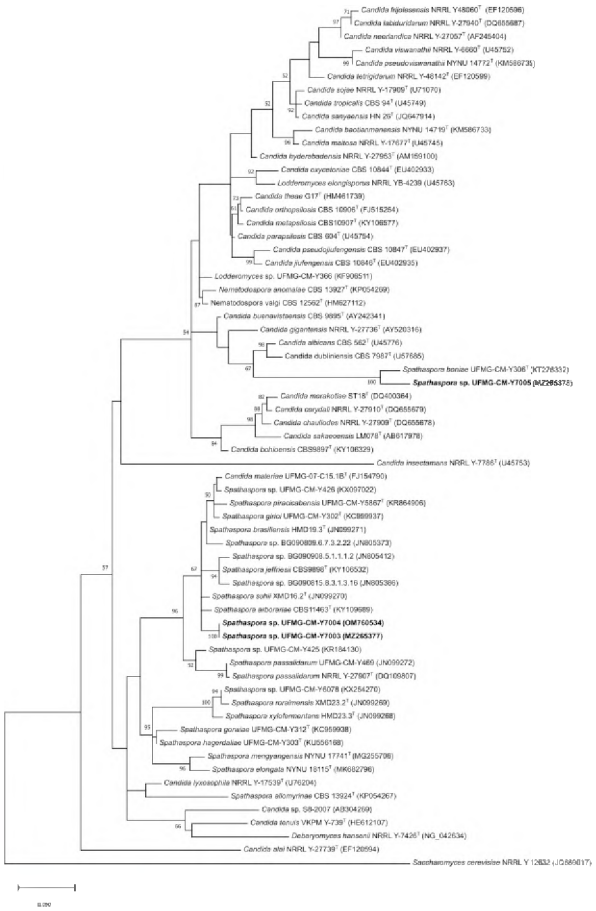

Figure 8. Phylogenetic placement of a candidate for a new species of *Spathaspora* based on the D1/D2 domains of the LSU rRNA gene sequences. The tree was reconstructed by maximum-likelihood analysis of 532 aligned positions using the Tamura-Nei substitution model with a gamma rate distribution and invariant sites. Bootstrap values were determined from 1000 pseudoreplicates. Bar, 0.05 substitutions per site.

Table 1. Identification of possible novel yeast species isolated in Amazonian Rainforest sites based on the sequences of the ITS region and D1/D2 domains of the large subunit of the rRNA gene.

Species	Strains (UFMG-CM-)	Related species (GenBank access D1/D2-ITS)	Identity D1/D2-ITS	Query cover (%) D1/D2-ITS	GenBank access D1/D2-ITS
Candida sp. 1	Y6924	*Candida tammaniensis* KY106792/*Candida cerambycidarum* NR_111392	647/667(97%)/ 585/586(99%)	98/100	OM089792/ OM089793
Candida sp. 2	Y6942	*Candida sanyaensis* MG833302/*Candida sojae* MK394120	555/557(99%)/ 709/729(97%)	100/100	OM349077/ OM349078
Candida sp. 3	Y6950, Y6953, Y6954, Y6955, Y6956, Y6957, Y6958, Y6959, Y6960, Y6961, Y6962, Y6963	*Candida nivariensis* MH545923	1115/1202(93%)	90	OM802403
Candida sp. 4	Y6947	*Candida insectorum* JN544058/AB365476	533/543(98%) 683/726(94%)	99/100	OM530522/ OM530523
Candida sp. 5	Y6938, Y6996	*Torulaspora globosa* CP059251 / *Ogataea parapolymorpha* CP080316	570/601(95%)/ 444/521(85%)	91/67	OM530526/ OM530525
Candida sp. 6	Y6939	*Saccharomyces eubayanus* CP064161 / *Vanderwaltozyma yarrowii* KY105799	561/593(95%)/ 294/311(95%)	100/40	OM530528/ OM632680
Cyberlindnera sp. 1	Y6925	*Cyberlindnera japonica* KY107369 / *Candida easanensis* KY102065	586/589(99%)/ 567/593(96%)	100/99	OM530244/ OM530245
Cyberlindnera sp. 2	Y6923	*Cyberlindnera maritima* KY106560/KY102194	579/588(98%) / 525/557 (94%)	100/100	OM089790/ OM089789
Kazachstania sp. 1	Y6926, Y6931, Y6940, Y6949, Y6997, Y6998	*Kazachstania martiniae* KY107933 / KY103655	581/589(99%)/ 635/645(98%)	100/100	OM530249/ OM530250
Kazachstania sp. 2	Y6927, Y6929, Y6930, Y6994, Y6995	*Kazachstania yasuniensis* NG_067774 / HG934853	585/586(99%)/ 608/612(99%)	100/100	OM530260/ OM530262

Kazachstania sp. 3	Y6928, Y6943	*Kazachstania africana* HE650828	1093/1200(91%)	96	OM845237
Kazachstania sp. 4	Y6932	*Kazachstania servazzii* KX987246	723/739(98%)		OM570228 / OM570227
Kurtzmaniella sp.	Y7001	*Kurtzmaniella quercitrusa* MK394107	704/730(96%)/ 699/730(96%)	100/100	OM760529 / OM760530
Pichia sp.	Y6933, Y6934, Y6935, Y6936, Y6937	*Pichia manshurica* MK394164 / FM199959	548/627(87%)/ 242/274(88%)	95/58	OM089796/ OM214537
Saccharomycopsis sp.	Y6991	*Saccharomycopsis amapae* KY106288 / *Saccharomycopsis fibuligera* MK497048	611/660(93%)/ 557/636(88%)	97/83	MZ682119/ OM214539
Scheffersomyces sp.	Y6944, Y6945, Y6946	*Scheffersomyces queiroziae* NG_059012 / *Scheffersomyces coipomoensis* MT277139	579/591(98%)/ 677/688(98%)	100/99	OM089802/ OM089801
Spathaspora sp. 1	Y7003, Y7004	*Spathaspora arborariae* KY109689 / *Spathaspora suhii* JN099270	553/564(98%)/ 729/770(95%)	100/96	MZ265377/ OM095307
Spathaspora sp. 2	Y7005	*Spathaspora boniae* MH299811	531/548(97%)/ 732/750(98%)	100/100	MZ265378/ OM095309
Spencermartinsiella sp. 1	Y7010, Y7012, Y7013, Y7014	*Spencermartinsiella ligniputridi* KY109693 / *Spencermartinsiella cellulosicola* NR_151783	578/594(97%)/ 493/556(89%)	100/72	OM095322/ OM214540
Spencermartinsiella sp. 2	Y6951, Y6952	*Spencermartinsiella cellulosicola* NG_055207/ NR_151783.	568/572(99%) 526/563(93%)	74/80	OM535826/ OP108370
Sugiyamaella sp. 1	Y6964, Y6972	*Sugiyamaella carassensis* KX550111	702/774(91%)/ 685/773(89%)	100/100	MZ264216/ MZ264217
Sugiyamaella sp. 2	Y6966, Y6968, Y6967, Y6969, Y6970, Y6971, Y6973, Y6974, Y6975, Y6976, Y6977, Y6988	*Sugiyamaella castrensis* KY106390 / *Sugiyamaella paludigena* KX609757	629/656(96%)/ 457/510(90%)	92/91	OL756104/ OL756103
Sugiyamaella sp. 3	Y6980,	*Sugiyamaella*	770/843(91%)/	100/100	MZ265324/

	Y6981, Y6985	*lignohabitans* CP014503	562/640(88%)		MZ265325
Sugiyamaella sp. 4	Y6982, Y6983, Y6984, Y6986	*Sugiyamaella bonitensis* KT006005	651/695(94%)/ 576/647(89%)	91/90	MZ265342/ MZ265348
Sugiyamaella sp. 5	Y6987	*Sugiyamaella trypani* MK387312 / *Sugiyamaella ayubii* NR_155796	493/511(96%)/ 405/473(86%)	92/65	OL756107/ OL756109
Sugiyamaella sp. 6	Y6978, Y6979	*Sugiyamaella americana* / *Sugiyamaella carassensis*			OL756105 / OL756108
Torulaspora sp.	Y6992	*Torulaspora globosa* CP059251 / *Torulaspora maleeae* KY105663	754/772(98%)/ 739/752(98%)	99/100	MZ254720/ MZ254721
Vanderwaltozyma sp.	Y6941, Y7006, Y7007, Y7008	*Vanderwaltozyma yarrowii* KY110004 / KY105799	601/630(95%)/ 504/600(84%)	83/84	OM095314/ OM095316
Wickerhamomyces sp.	Y6965, Y7185, Y7186, Y7187, Y7188, Y7189, Y7190, Y7191, Y7192, Y7193, Y7194, Y7195, Y7196, Y7197, Y7198, Y7199, Y7200	*Wickerhamomyces edaphicus* AB247371 / *Candida nivariensis* KP068747	580/581(99%)/ 584/704(83%)	96/100	OP292296/ OP292297
Yueomyces sp.	Y7009, Y7011	*Yueomyces sinensis* KP866235	1064/1125(95%)	98	OM845233