Antonie van Leeuwenhoek
DOI 10.1007/s10482-015-0546-8

ORIGINAL PAPER

Xylitol production by yeasts isolated from rotting wood in the Galápagos Islands, Ecuador, and description of *Cyberlindnera galapagoensis* f.a., sp. nov.

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Received: 8 April 2015 / Accepted: 22 July 2015

Abstract This study evaluated D-xylose-assimilating yeasts that are associated with rotting wood from the Galápagos Archipelago, Ecuador, for xylitol production from hemicellulose hydrolysates. A total of 140 yeast strains were isolated. Yeasts related to the clades *Yamadazyma, Kazachstania, Kurtzmaniella, Lodderomyces, Metschnikowia* and *Saturnispora* were predominant. In culture assays using sugarcane bagasse hemicellulose hydrolysate, *Candida tropicalis* CLQCA-24SC-125 showed the highest xylitol production, yield and productivity (27.1 g L^{-1} xylitol, $Y^{xyl}_{p/s} = 0.67$ g g^{-1}, $Q_p = 0.38$ g L^{-1}. A new species of *Cyberlindnera,* strain CLQCA-24SC-025, was responsible for the second highest xylitol production (24 g L^{-1}, $Y^{xyl}_{p/s} = 0.64$ g g^{-1}, $Q_p = 0.33$ g L^{-1} h^{-1}) on sugarcane hydrolysate. The new xylitol-producing species *Cyberlindnera galapagoensis* f.a., sp. nov., is proposed to accommodate the strain CLQCA-24SC-025^T (=UFMG-CM-Y517^T; CBS 13997^T). The MycoBank number is MB 812171.

Keywords Yeasts · Xylitol · Galápagos archipelago · D-xylose conversion · Sugarcane bagasse hemicellulose hydrolysate · *Cyberlindnera galapagoensis* sp. nov.

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Introduction

Xylitol, a five-carbon sugar alcohol, is not metabolised by microorganisms of the oral microbiota and has anticariogenic and cariostatic properties (Winkelhausen and Kuzmanova 1998; Mohamad et al. 2015). This polyol is important in the cure and prevention of a number of diseases. Xylitol metabolism is insulin independent; it is processed via the pentose-monophosphate shunt, and it is a sugar substitute that has been approved for clinical parenteral nutrition (Schneider et al. 2014). Because of its sweetening strength that is similar to sucrose and higher than ordinary polyols and its reduced caloric value and low insulin release, xylitol is considered a good sugar substitute in controlled diets (Mussatto and Roberto 2002; Mohamad et al. 2015). In addition, studies have shown that xylitol prevents osteoporosis, haemolytic anaemia and acute otitis (Mussatto and Roberto 2002).

Published online: 29 July 2015

Xylitol can be produced by the chemical reduction of D-xylose derived mainly from lignocellulose biomass hydrolysates (Chen et al. 2010; da Silva and Chandel 2012; Mohamad et al. 2015). However, the requirement for several purification steps to separate xylitol from other polyols and sugars during the chemical process increases the production cost. The alternative, economical production of xylitol can be performed by bacteria, filamentous fungi, yeasts or their purified enzymes, all of which are capable of reducing D-xylose to xylitol as the first step in D-xylose metabolism. In general, yeasts are considered the best xylitol producers (Sampaio et al. 2008); these microorganisms metabolise D-xylose primarily through a two-stage oxidative-reductive pathway where D-xylose is reduced to xylitol by an NAD(P)H-dependent xylose reductase (XR), and the xylitol is then oxidised to D-xylulose by an NAD^+-dependent xylitol dehydrogenase (XDH) (da Silva et al. 1996). D-xylulose is then phosphorylated by xylulokinase (XK) to produce D-xylulose-5-phosphate, which can be converted into pyruvate via a connection between the pentose phosphate and the glycolytic pathways (Winkelhausen and Kuzmanova 1998; da Silva and Felipe 2006). The redox equilibrium between the reactions catalysed by XR and XDH favours xylitol production (Winkelhausen and Kuzmanova 1998). Although XRs that utilise NAD(P)H exist, NADPH is the preferred cofactor in most of the known D-xylose-fermenting yeasts, and XDH requires NAD^+. This leads to the accumulation of $NADP^+$ and NADH, respectively, in the oxidoreductive process. Whereas $NADP^+$ can be reduced by recycling D-fructose-6-phosphate through the oxidative pentose phosphate pathway (PPP) during pentose metabolism (Bruinenberg et al. 1983), NADH is mainly oxidised to NAD^+ by oxygen in the respiratory chain. Under oxygen-limiting conditions, NAD^+ is not efficiently regenerated, and xylitol is accumulated (Gírio et al. 2010). Thus, yeasts that have strictly NADPH-dependent XR produce xylitol as the major product of D-xylose conversion under oxygen-limiting conditions (da Silva et al. 1996; da Silva and Chandel 2012).

For the production of xylitol via biotechnology, hemicellulose hydrolysates obtained from lignocellulose materials can be used as substrates (Arruda et al. 2011; Mohamad et al. 2015). Lignocellulose materials are composed of the carbohydrate polymers cellulose (35–50 %) and hemicellulose (20–35 %) and an amorphous tridimensional macromolecule, lignin (10–25 %) (Saha 2003). The hemicellulose component is a heterogeneous class of polymers that includes xylan, a homopolymer formed by D-xylose units and this pentose is the most abundant hemicellulose backbone (Saha 2003; Gírio et al. 2010). Depolymerisation of the cellulose and hemicellulose fractions leads to the release of their constituent sugars, mainly glucose from cellulose and D-xylose from hemicellulose. Once released from the hemicellulose, D-xylose can be converted to xylitol (Saha 2003). However, despite the existence of xylitol-producing yeasts such as *Candida tropicalis*, *Cyberlindnera saturnus, Debaryomyces hansenii, Meyerozyma guilliermondii* and *Pachysolen tannophilus* (da Silva and Afschar 1994; Leathers and Gupta 1997; Converti et al. 1999; Kamat et al. 2013), one of the greatest challenges in xylitol production by biotechnology processes is the availability of strains able to produce high yields of this polyol in an industrial context. D-xylose-assimilating yeasts are closely associated with rotting wood in nature, and several new yeasts have been isolated from this substrate in ecosystems with high biodiversity rates (Cadete et al. 2012; Morais et al. 2013a, b).

In this study, we investigated three islands in the Galápagos Archipelago (Ecuador) for yeast species that were able to assimilate D-xylose and convert this pentose to xylitol using a D-xylose supplemented medium or sugarcane bagasse hemicellulose hydrolysate. We also describe a novel yeast species, *Cyberlindnera galapagoensis* f.a., sp. nov., able to produce xylitol.

Materials and methods

Collection area and yeast isolation

The Galápagos Archipelago belongs to Ecuador and is located approximately 1000 km from the continental coast. The archipelago is home to some of the highest levels of endemism anywhere on the planet, and it is a habitat that contains peculiar fauna and flora. The vegetation is arid in the lower areas of the islands, and it is a subtropical humid forest in the upper parts (Neill and Jorgensen 1995). The average temperature ranges from 17 to 25 °C throughout the year, and the precipitation and rain distribution depends on Pacific Ocean currents (Galápagos National Park 2012).

Thirty-five rotting-wood samples of approximately 10 grams each were collected on three islands of the Galápagos Archipelago in October 2009. The field collections were made according to the rules and permission of Ecuador government. On Floreana Island, 12 samples were collected at the Turtles Protection Station. On Isabela Island, four samples were gathered near the volcano "Sierra Negra". On Santa Cruz Island, 19 samples were collected in "Puerto Ayora" and in the craters of "Los Gemelos" and "Media Luna"; the latter point is at the highest altitude on the island.

The wood samples (~10 g) were stored in sterile plastic bags and transported to the laboratory under refrigeration. Approximately 1 gram of each sample was separately placed in 125 mL Erlenmeyer flasks with 20 mL of the following media: YNB-D-xylose (yeast nitrogen base, 0.67 %; D-xylose, 2 % and chloramphenicol, 0.02 %), YNB-xylan (yeast nitrogen base, 0.67 %; xylan, 2 % and chloramphenicol, 0.02 %) or YNB-CMC (yeast nitrogen base, 0.67 %, carboxymethylcellulose, 1 %; cellobiose, 0.05 % and chloramphenicol, 0.02 %). The D-xylose, xylan, CMC and YNB solutions were sterilised separately. The flasks were incubated at 25 °C on an orbital shaker (New Brunswick, Edison, NJ, USA) at 150 rpm for 3–15 days. Upon detection of cell growth, 0.5 mL was transferred to a tube containing 5 mL of sterile YNB-D-xylose, YNB-xylan or YNB-CMC media. The tubes were incubated on an orbital shaker under the same conditions described above. After growth was detected, one loopful from each tube was streaked on YNB-D-xylose, YNB-xylan or YNB-CMC agar media (Cadete et al. 2012). The plates were incubated at 25 °C until colonies developed. The different yeast morphotypes were purified by restreaking on yeast extract-malt extract agar (YMA—glucose, 1 %; peptone, 0.5 %; yeast extract, 0.3 %; malt extract, 0.3 %; agar, 2 %) and cryopreserved with GYMP broth (glucose, 2 %; yeast extract, 0.5 %; malt extract, 1 %; monobasic sodium phosphate, 0.02 % and glycerol) in a ultra-freezer at −80 °C.

Yeast identification

Yeasts were preliminarily grouped according to their morphological characteristics and their growth responses on different carbon and nitrogen sources (Kurtzman et al. 2011). Physiology-based groupings were confirmed by PCR fingerprinting using the Intron Splice Site primer EI1 (CTGGCTTGGTGTATGT) (de Barros et al. 1996). Conspecific isolates generally produce identical banding profiles using this PCR-fingerprinting technique (Rosa et al. 2007). Yeast strains with identical PCR fingerprinting patterns were grouped and putatively considered to belong to the same species (Cadete et al. 2012). At least one representative strain from each EI1 group was subjected to sequence analysis of the D1/D2 region of the large subunit (LSU) of the rRNA gene and the ITS-5.8S rRNA gene region, directly from whole cells, as described previously (Lachance et al. 1999). The amplified DNA was concentrated, cleaned and sequenced in an ABI3130 (Life Technologies, Carlsbad, CA, USA) automated sequencing system. The sequences were assembled, edited and aligned with the program MEGA6 (Tamura et al. 2013). The existing sequences for other yeasts were retrieved from GenBank. Phylogenetic placement of the novel species was based on a maximum likelihood analysis of the sequences of the D1/D2 domains of the large subunit rRNA gene, using the General Time Reversible model. Bootstraps were obtained from 100 iterations using 532 aligned nucleotide positions.

Screening of D-xylose-assimilating and xylitol-producing yeasts

Yeasts showing positive results for D-xylose assimilation were then grown in YNB-D-xylose medium for 48 h. The cultures were diluted in sterile distilled water to approximately 2×10^8 cells mL^{-1} and 200 µL of this solution was inoculated into tubes containing 1.8 mL of YPX broth (yeast extract, 1 %; peptone, 2 %; D-xylose, 3 %) and incubated at 25 °C and 200 rpm for 48 h. Samples were taken at 0, 24 and 48 h to determine the cell concentration, the D-xylose consumption and the xylitol production. Yeasts showing the best D-xylose consumption and the highest xylitol production in YPX broth were assayed for their ability to produce xylitol in supplemented medium containing D-xylose and, subsequently, in sugarcane bagasse hemicellulose hydrolysate.

Xylitol production in D-xylose supplemented medium

Yeast inocula were prepared in 250 mL Erlenmeyer flasks with 100 mL of YPX medium containing D-

xylose (3 %), rice bran extract (2 %), $(NH_4)_2SO_4$ (0.2 %) and $CaCl_2{\cdot}2H_2O$ (0.01 %), with continuous shaking at 200 rpm and 30 °C for 24 h. Subsequently, the cells were harvested by centrifugation at 2600×*g* for 20 min, rinsed twice with distilled water and re-suspended in the culture media according to the concentrations specified for each assay. Cell concentration was determined with a spectrophotometer (Beckman Coulter, Pasadena, CA, USA) by the absorbance at 600 nm, using a previously generated calibration curve (dry weight × Abs_{600}) (Cadete et al. 2012).

For each yeast strain, the experiments were performed in triplicate in 250 mL Erlenmeyer flasks that contained 100 mL of D-xylose supplemented medium (D-xylose, 5 %; rice bran extract, 2 %; $(NH_4)_2SO_4$, 0.2 %; $CaCl_2{\cdot}2H_2O$, 0.01 %), pH 5.5. The initial cell concentration was 1 g L^{-1}. Flasks were incubated in an orbital shaker at 30 °C and 200 rpm for 72 h, and monitored by sampling at 0, 12, 24, 48 and 72 h. Yeasts showing the best D-xylose consumption and the highest xylitol yields ($Y^{xyl}_{p/s}$) were evaluated for their ability to produce xylitol in sugarcane bagasse hemicellulose hydrolysate.

Xylitol production in sugarcane bagasse hemicellulose hydrolysate

Sugarcane bagasse was supplied by Usina Costa Pinto of the COSAN group (Piracicaba, São Paulo, Brazil). The hydrolysis was carried out using diluted H_2SO_4 (100 mg of H_2SO_4 per gram of dry matter) and a solid/liquid ratio of 1:10, at 121 °C for 20 min in a 350 L steel reactor. After hydrolysis, the resulting solid material was removed by filtration and the hemicellulose hydrolysate was concentrated in a 30 L evaporator at 70 ± 5 °C to increase its original D-xylose concentration five-fold. To reduce inhibitors, the hydrolysate was detoxified as described by Cadete et al. (2012); the pH was raised to 7.0 with calcium oxide and then decreased to pH 5.5 with phosphoric acid, followed by the addition of activated charcoal (2.5 % w/v) and incubation at 200 rpm and 30 °C for 1 h. After each procedure, precipitates were removed by vacuum filtration. The sugar composition of the hydrolysate before detoxification and autoclaving was 61.7 g L^{-1} D-xylose, 5.8 g L^{-1} glucose and 4.7 g L^{-1} L-arabinose. The treated hydrolysate was autoclaved at 111 °C for 15 min under 0.5 atm. The average hydrolysate used as the culture medium was composed of 49.7 g L^{-1} D-xylose, 4.8 g L^{-1} glucose, 3.4 g L^{-1} L-arabinose, 1.6 g L^{-1} acetic acid, 0.17 g L^{-1} furfural and 0.03 g L^{-1} hydroxymethylfurfural, at pH 5.0.

Experiments were carried out at an initial cell concentration of 1 g L^{-1} in 250 mL Erlenmeyer flasks containing 100 mL of supplemented hemicellulose hydrolysate (rice bran extract, 2 %; $(NH_4)_2SO_4$, 0.2 %; $CaCl_2{\cdot}2H_2O$, 0.01 %) at pH 5.5 on a rotary shaker at 200 rpm, 30 °C for 120 h. Samples were taken at 0, 12, 24, 48, 72, 96 and 120 h and stored at −20 °C until analysis. All experiments were performed in triplicate.

Determination of sugars and metabolites

Samples were centrifuged at 2000×*g* for 15 min and the supernatant was diluted and filtered using a Sep-Pak C18 (Millipore, MA, USA) filter. Sugars (glucose, D-xylose and L-arabinose) and metabolites (xylitol, glycerol, ethanol and acetic acid) were determined with an Agilent 1200 series (Agilent Technologies, Santa Clara, CA, USA) HPLC system using a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column at 45 °C with a sample injection volume of 20 µL, an RID-6A refractive index detector, a mobile phase of 0.01 N H_2SO_4 and a flow rate of 0.6 mL min^{-1}.

Determination of toxic compounds

Samples were pre-filtered through a Minisart 0.22 micrometre membrane (Millipore) and analysed by HPLC to determine the inhibitors 5-hydroxymethylfurfural and furfural under the following conditions: a Zorbax Eclipse Plus C18 (3.5 µm, 10 cm × 4.6 mm ID) column with a UV–Vis detector (276 nm), a solution of acetonitrile/H_2O (1:8) and 1 % acetic acid as eluent, a flow rate of 0.8 mL min^{-1} and an injection volume of 20 µL.

Conversion parameters calculation

The parameters $Y^{xyl}_{p/s}$ (g g^{-1}, xylitol yield), Q_p (g L^{-1} h^{-1}, xylitol productivity), $Y_{x/s}$ (g g^{-1}, cell biomass yield), η (%, conversion efficiency), D-xylose and/or glucose consumption (%), xylitol and ethanol concentration (g L^{-1}) were experimentally determined according to the procedures described by

Cadete et al. (2012). D-xylose and/or glucose consumption (%) was determined as a percentage of the initial sugar concentration. The conversion efficiency (%) was determined as a percentage of the maximum theoretical xylitol yield (0.917 g xylitol g^{-1} D-xylose consumed) (Barbosa et al. 1988).

Results and discussion

Yeast isolation and identification

In this work, a total of 140 yeasts belonging to 15 genera and 35 species were obtained from 35 rotting-wood samples collected on three islands of the Galápagos Archipelago. Table 1 shows the results of yeast identification, the occurrence of each species by isolation site and the number of isolates cultured in each medium. *Barnettozyma californica, C. tropicalis*, *Candida natalensis, C. sinolaborantium* and *Kazachstania unispora* were the most frequently isolated species in this study. *B. californica* appears to have a worldwide distribution and is associated primarily with soil from several locations around the world (Kurtzman et al. 2011). Strains of *C. tropicalis* have been obtained from fruits, cacti, soil, water, clinical specimens and rotting wood (Kurtzman et al. 2011; Cadete et al. 2012; Morais et al. 2013a, b). *C. natalensis* has been previously isolated from soil and rotting wood (Kurtzman et al. 2011; Cadete et al. 2012), whereas *C. sinolaborantium* is associated with the gut of a fungus beetle and with the gut and the surface of a cerambycid larva (Kurtzman et al. 2011). Strains of *K. unispora* are associated with naichin, kefir grains, faeces and cheese (Kurtzman et al. 2011). Overall, most of the known yeast species found in this study have previously been linked to terrestrial environments, such as soil, flowers, fruit, rotting wood, beetle guts and insects (Kurtzman et al. 2011).

Screening of D-xylose-assimilating and xylitol-producing yeasts

In this study, 99 yeast strains assimilated D-xylose as the sole carbon source, corresponding to 70.7 % of the total number of isolates. All yeast strains obtained from YNB-xylan medium were able to assimilate this pentose. From the isolates grown in YNB-CMC, 19 assimilated D-xylose (82.6 %), whereas 63 strains (63 %) obtained from YNB-D-xylose assimilated this pentose. Several isolates (37 %) from YNB-D-xylose were not able to assimilate D-xylose. Growth of filamentous fungi that produce enzymes able to degrade the cellulose present in rotting wood could explain the isolation of these species. These enzymes could liberate glucose from cellulose and other nutrients, leading to the growth of yeasts unable to assimilate D-xylose in the culture medium. Rao et al. (2008) isolated 374 yeast strains from tree bark and rotten fruit cultured in YMA medium, of which 27 assimilated D-xylose (representing the 11.3 % of the total). A similar study by Guo et al. (2006) reported 45 (16.4 % of the total) D-xylose-assimilating strains among 274 yeast strains grown in YPX (yeast extract, peptone and D-xylose) medium. The D-xylose-assimilating species in both studies corresponded to species belonging to the genera *Candida, Trichosporon, Geotrichum* and *Pichia*.

To evaluate xylitol production, the D-xylose-assimilating yeasts were cultured in YPX broth. In this assay, 50 strains belonging to 15 different species produced xylitol (Table 1). Among these species, *C. tropicalis* has previously been studied for the production of ethanol and primarily xylitol from lignocellulose residues (Rao et al. 2006; Arrizon et al. 2012). *Candida intermedia* is known to exhibit high D-xylose transport capacity (Leandro et al. 2006), but low xylitol production in a defined mineral medium containing D-xylose as the carbon source (Gárdonyi et al. 2003). *Scheffersomyces stipitis* is able to produce high amounts of ethanol associated with low xylitol production from D-xylose in fermentation processes (Cadete et al. 2012). For the remaining species identified as xylitol producers in the present study, no previous studies showing this activity were found. These novel xylitol-producing species were *B. californica, C. amphixiae, C. naeodendra, C. pseudointermedia, C. sinolaborantium, D. nepalensis, G. geotrichum*, *Cyberlindnera* sp., *Tr. coremiiforme, Tr. laibachii, W. anomalus* and *Y. mexicana*.

Xylitol production in D-xylose supplemented medium

The seven strains showing the best D-xylose consumption and highest xylitol production (higher than 6 g L^{-1}) in YPX broth were tested for xylitol production and growth in D-xylose (5 %) supplemented

Table 1 Identification, occurrence, D-xylose assimilation and xylitol production of yeasts isolated in the Galápagos Islands

Yeast species	Sampled medium									D-xylose assimilation	Xylitol production in YPX[d]
	CMC			YNB-D-xylose			YNB-xylan				
	F[a]	SC[b]	I[c]	F	SC	I	F	SC	I		
Barnettozyma californica		1				5		2	1		+
Candida albicans (*Lodderomyces* clade)				2[e]			2			+	−
C. amphixiae (*Yamadazyma* clade)	1									+	3.83
C. dendronema (*Yamadazyma* clade)	2									+	−
C. humilis (*Kazachstania* clade)					1					−	−
C. intermedia (*Metschnikowia* clade)	1				1					+	4.35
C. naeodendra (*Yamadazyma* clade)	1									+	3.43
C.(*Kurtzmaniella*) *natalensis*	2			5			3			v[f]	−
C. parapsilosis (*Lodderomyces* clade)					1					+	−
C. pseudointermedia (*Metschnikowia* clade)	3				1					+	6.55
C. sinolaborantium				1	6					+	4.83−7.81
C. silvae (*Saturnispora* clade)	2			4						−	−
C. tropicalis (*Lodderomyces* clade)		1		14	4		2	1		+	3.18−6.78
C. trypodendroni (*Yamadazyma* clade)		1			5					+	−
Cryptococcus flavescens (*Bulleromyces* clade)	1					1				+	−
Cr. humicola (*Bulleromyces* clade)					1					+	−
Cr. laurentii (*Bulleromyces* clade)					1	1			1	+	−
Cyberlindnera saturnus		1								−	−
C. galapagoensis sp. nov.[g]					1					+	6.19
Debaryomyces hansenii								1		+	−
D. nepalensis		1			1		1	1		+	4.88
Galactomyces geotrichum		1			1		2			+	5.54
Geotrichum silvicola					1					+	−
Kazachstania exigua					1					+	−
K. unispora				13	2					−	−
Pichia manshurica				6						−	−
P. terricola				4						v	−
Scheffersomyces stipitis					2					+	3.73–7.72
Torulaspora delbrueckii				6						−	−
Trichosporon coremiiforme				1						+	3.78
Tr. jirovecii		1		2						+	−
Tr. laibachii	1									+	2.96
Tr. multisporon	1									+	−
Wickerhamomyces anomalus				2						+	4.28
Yamadazyma mexicana	1					3				+	5.58–5.82
Total (140)	17	6		60	35	5	12	4	1		

[a] Occurrence of yeast species restricted to Floreana Island

[b] Occurrence of yeast species restricted to Santa Cruz Island

[c] Occurrence of yeast species restricted to Isabela Island

[d] YPX broth (yeast extract, 1 %; peptone, 2 %; D-xylose, 3 %)

[e] Number of samples in which the yeast was isolated

[f] Variable

[g] Novel yeast species

medium (Table 2). The results revealed consumption of D-xylose ranging from 42.18 to 99.22 % for the tested strains. *C. tropicalis* CLQCA-24F-125 showed the highest consumption of this pentose followed by *C. pseudointermedia* CLQCA-24F-113 and *Cyberlindnera* sp. CLQCA-24SC-025 after 72 h of incubation. Xylitol production was observed for all of the yeasts that were tested. Both strains of *C. sinolaborantium,* CLCQA-24F-127 and 24SC-027, showed an equal xylitol yield (0.25 g g^{-1}) in this assay. *C. sinolaborantium* has been isolated from beetle gut and the larvae of cerambycids (Cerambycidae) in Panama and USA, and this is the first report of the isolation of this species from rotting wood. The insects associated with the wood are possibly the vectors of this yeast. The biotechnology and food applications and the clinical importance of this species are unknown (Lachance et al. 2011). For this reason, *C. sinolaborantium* was selected for evaluation in assays with sugarcane hemicellulose hydrolysate, despite its low xylitol production compared with the other strains tested in D-xylose supplemented medium.

The strain *C. tropicalis* CLQCA-24F-125 showed promising xylitol production under the conditions tested, considering its high xylitol concentration (25.63 g L^{-1}), productivity (Q_p = 0.34 g L^{-1} h^{-1}), conversion efficiency (η = 73.66 %) and yield ($Y^{xyl}_{p/s}$ = 0.67 g g^{-1}). Several authors have already indicated that *C. tropicalis* is a remarkable xylitol producer. Da Silva and Afschar (1994) assessed xylitol production from D-xylose by this species. Conversion efficiencies between 77 and 80 % and xylitol productivity equal to 0.37 g L^{-1} h^{-1} were observed by these authors. De Mello (2009) observed a xylitol production equal to 32.9 g L^{-1} in 72 h with *C. tropicalis* cultured in a complex medium containing 50 g L^{-1} D-xylose supplemented with peptone and urea. The results of that assay were as follows: xylitol yield (0.65 g g^{-1}), productivity (0.35 g L^{-1} h^{-1}) and conversion efficiency (61 %). For the *C. tropicalis* CLQCA-24F-125 tested in this study, similar values of xylitol yield and productivity were achieved, and a greater efficiency of D-xylose to xylitol was observed. Cadete et al. (2012) found *Scheffersomyces amazonensis* was the best xylitol-producing yeast strain. This species showed the maximum xylitol production (27.8 g L^{-1}) and yield (0.67 g g^{-1}) after 48 h of growth in a complex medium formulated with

Table 2 Xylitol yield [$Y^{xyl}_{p/s}$ (g g^{-1})], xylitol productivity [Q_p (g L^{-1} h^{-1})], cell biomass yield [$Y_{x/s}$ (g g^{-1})], conversion efficiency [η (%)], D-xylose consumption (%), cell, xylitol and ethanol concentration (g L^{-1}) in 5 % D-xylose supplemented medium assays

Yeast species and strain	$Y^{xyl}_{p/s}$ (g g^{-1})[a]	Q_p (g L^{-1} h^{-1})[b]	$Y_{x/s}$ (g g^{-1})[c]	η (%)[d]	Cells (g L^{-1})	D-xylose consumption (%)[e]	Xylitol (g L^{-1})	Ethanol (g L^{-1})	Time (h)[f]
Barnettozyma californica CLQCA-24SC-143	0.09	0.02	0.26	9.32	5.83	46.94	1.86	0.61	72
Candida pseudointermedia CLQCA-24F-113	0.39	0.27	0.14	43.06	4.99	87.51	13.90	5.36	48
C. sinolaborantium CLQCA-24F-127	0.25	0.08	0.30	27.21	7.61	55.50	5.86	0.75	72
C. sinolaborantium CLQCA-24SC-027	0.25	0.06	0.33	27.85	6.23	42.18	4.41	0.13	72
C. tropicalis CLQCA-24F-125	0.67	0.34	0.17	73.66	6.85	99.22	25.63	0.02	72
Cyberlindnera galapagoensis sp. nov. CLQCA-24SC-025	0.50	0.23	0.14	55.47	5.22	85.54	17.01	0.92	72
Scheffersomyces stipitis CLQCA-24SC-321	0.02	0.005	0.36	1.87	8.53	52.87	0.36	3.81	72

[a] $Y^{xyl}_{p/s}$ (g g^{-1}): xylitol yield: correlation between xylitol (ΔP) produced and D-xylose (ΔS) consumed

[b] Q_p (g L^{-1} h^{-1}): xylitol productivity: ratio of xylitol concentration (g L^{-1}) and time (h) of maximum xylitol production

[c] $Y_{x/s}$ (g g^{-1}): cell biomass yield: correlation between cell biomass produced and D-xylose consumed

[d] η (%): conversion efficiency: percentage of the maximum theoretical xylitol yield (0.917 g xylitol/g D-xylose)

[e] D-xylose consumption (%): percentage of initial D-xylose consumed

[f] Time when the maximum xylitol production (g L^{-1}) value was attained or time of the end of the experiment

50 g L^{-1} D-xylose and supplemented with peptone and yeast extract. In this study, *C. tropicalis* CLQCA-24F-125 had a similar xylitol production and equal xylitol yield after 72 h of growth.

The strain with the second highest xylitol production was the new species *Cyberlindnera* sp. CLQCA-24SC-025, with a xylitol yield and conversion efficiency equal to 0.5 g g^{-1} and 55.47 %, respectively. The productivity achieved by this species was lower than that obtained from *C. pseudointermedia* CLQCA-24F-113, although the yield and efficiency of *C. pseudointermedia* was lower than that produced by the new species. However, a decrease in the xylitol concentration after 48 h was observed for *C. pseudointermedia* (data not shown), probably resulting from xylitol assimilation by the yeast as a consequence of D-xylose depletion in the medium (Cadete et al., 2012).

S*cheffersomyces stipitis* CLQCA-24SC-321 showed the lowest xylitol production among the strains tested in this study. Although *Sc. stipitis* is known to convert D-xylose into ethanol with high yield (Cadete et al. 2012), this activity was not observed in this study because the tested strain produced 3.81 g L^{-1} ethanol in 72 h. The highest ethanol production was shown by *C. pseudointermedia* CLQCA-24F-113, with 5.36 g L^{-1} in 48 h. *B.* The other species produced less than 1 g L^{-1} ethanol.

Xylitol production in sugarcane bagasse hemicellulose hydrolysate

Four strains with the highest D-xylose consumption values and xylitol yields in the previous assays were selected for testing for xylitol production in sugarcane bagasse hydrolysate (Table 3). *Candida tropicalis* had the highest xylitol production (27.12 g L^{-1}) in this assay. Additionally, this strain showed the best xylitol productivity (0.38 g L^{-1} h^{-1}) and yield (0.67 g g^{-1}) in hemicellulose hydrolysate, similar to the results achieved in D-xylose supplemented medium. Sugarcane bagasse hydrolysate was considered the best medium for xylitol production by yeast in a study by Arrizon et al. (2012). Those authors also reported high xylitol production for a *C. tropicalis* strain (0.29 g g^{-1}). Rao et al. (2006), evaluating *C. tropicalis* in an assay with sugarcane bagasse hydrolysate, obtained a xylitol yield (0.45 g g^{-1}) lower than that achieved in the present work. *Cyberlindnera* sp. showed the second highest xylitol production and reached 23.98 g L^{-1} after 72 h. *Cyberlindnera* sp. CLQCA-24SC-025 required a period of adaptation to the medium; during the first hours of growth, this species did not produce xylitol and the consumption of D-xylose was low (data not shown). The ability of *Cyberlindnera* sp. to consume 93.23 % of the D-xylose with a productivity and yield equal to 0.33 g L^{-1} h^{-1} and 0.64 g L^{-1}, respectively, without a concomitant increase in the cell density could be interesting in an industrial application. *Cyberlindnera saturnus*, the closest species to *Cyberlindnera* sp., was described by Kamat et al. (2013) as a xylitol-producing yeast using sugarcane bagasse hydrolysate as the culture medium. Those authors reported a xylitol yield of 0.51 g g^{-1} after 72 h for that yeast species. Comparing their results with those for *Cyberlindnera* sp. CLQCA-24SC-025, a higher xylitol yield was observed for the new species at the same time that maximum xylitol production occurred. *C. tropicalis* and *Cyberlindnera* sp. produced the maximum xylitol yield at 72 h of cultivation. After this time, xylitol started to decrease, indicating that it was consumed as a carbon source by the yeast (data not shown). *Candida pseudointermedia* and *C. sinolaborantium* showed a maximum xylitol production at 120 h, thus requiring a longer time to reach maximum xylitol production.

In this study, hemicellulose hydrolysate had a greater xylitol production than D-xylose supplemented medium, except for *C. tropicalis*. This result was similar to that obtained by Cadete et al. (2012) with four *Spathaspora* species, which also attained a higher xylitol concentration in hemicellulose hydrolysate compared to a complex medium. This behaviour can be explained by differences in the composition of each culture medium. In addition to D-xylose, sugarcane bagasse hydrolysates contain other sugars such as glucose and arabinose, as well as inorganic compounds such as manganese, iron, magnesium, nickel, calcium, sodium, potassium and chromium, and vitamins and amino acids (Arruda et al. 2011). All of these compounds could serve as macro and micronutrients for yeast growth by increasing the xylitol production. The addition of these supplements might accelerate the consumption of D-xylose and maximise xylitol production by providing essential precursors to cells.

The highest ethanol production was obtained after 120 h by *C. pseudointermedia* and *C. sinolaborantium*. However, xylitol was the main product attained

Table 3 Xylitol yield [$Y_{p/s}^{xyl}$ (g g^{-1})], xylitol productivity [Q_p (g L^{-1} h^{-1})], cell biomass yield [$Y_{x/s}$ (g g^{-1})], conversion efficiency [η (%)], D-xylose consumption (%), cell, xylitol and ethanol concentration (g L^{-1}) in sugarcane bagasse hemicellulose hydrolysate

Yeast species and strain	$Y_{p/s}^{xyl}$ (g g^{-1})[a]	Q_p (g L^{-1} h^{-1})[b]	$Y_{x/s}$ (g g^{-1})[c]	η (%)[d]	Cells (g L^{-1})	D-xylose consumption (%)[e]	Xylitol (g L^{-1})	Ethanol (g L^{-1})	Time (h)[f]
Candida pseudointermedia CLQCA-24F-113	0.39	0.13	0.26	42.94	11	99.19	15.55	4.05	120
C.sinolaborantium CLQCA-24F-127	0.49	0.16	0.27	53.80	11.17	95.71	19.48	4.08	120
C. tropicalis CLQCA-24F-125	0.67	0.38	0.26	73.50	11.07	99.08	27.12	2.50	72
Cyberlindnera galapagoensis sp. nov. CLQCA-24SC-025	0.64	0.33	0.15	70.58	5.96	93.23	23.98	3.36	72

[a] $Y_{p/s}^{xyl}$ (g g^{-1}): xylitol yield: correlation between xylitol (ΔP) produced and D-xylose (ΔS) consumed

[b] Q_p (g L^{-1} h^{-1}): xylitol productivity: ratio of xylitol concentration (g L^{-1}) and time (h) of maximum xylitol production

[c] $Y_{x/s}$ (g g^{-1}): cell biomass yield: correlation between cell biomass produced and D-xylose consumed

[d] η (%): conversion efficiency: percentage of the maximum theoretical xylitol yield (0.917 g xylitol/g D-xylose)

[e] D-xylose consumption (%): percentage of initial D-xylose consumed

[f] Time when the maximum xylitol production (g L^{-1}) value was attained or time of the end of the experiment

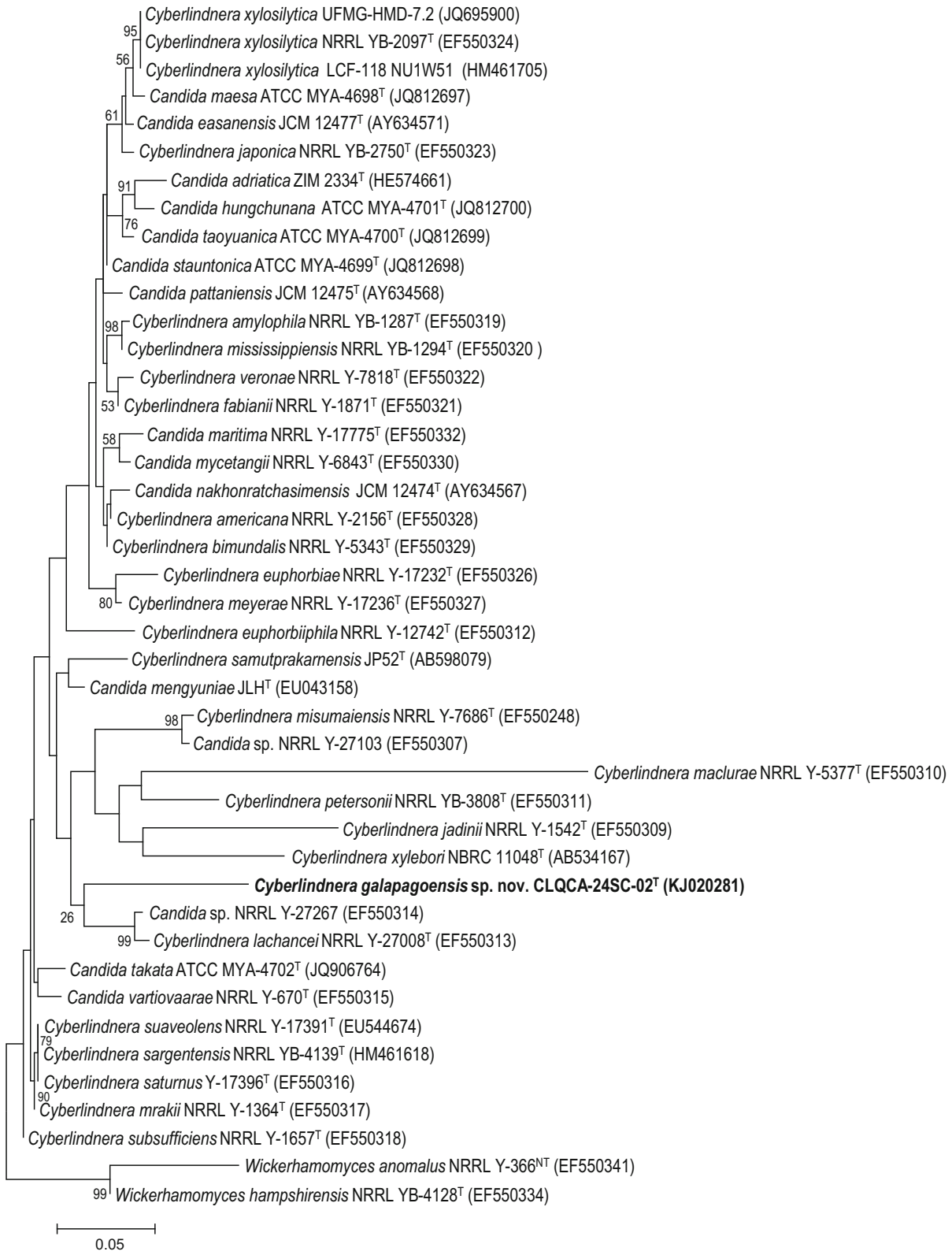
from the metabolism of the D-xylose present in sugarcane bagasse hydrolysate. Further studies are necessary to find the best conditions for xylitol production by these yeast species.

Description of *Cyberlindnera galapagosensis* f. a., sp. nov.

The yeast strain CLQCA-24SC-025 (GenBank accession number KJ020281) represents a new species of the genus *Cyberlindnera*. This species differs by 43 nucleotide substitutions and six indels in the D1/D2 region of the LSU rRNA gene from *Cyberlindnera lachancei*, its closest relative (Fig. 1). ITS sequences of the related species were not available for identity comparison. We propose the new species *Cyberlindnera galapagoensis* f.a., sp. nov. The mention *forma asexualis* (*f.a.*) is added as a reminder that a sexual state is not known (Lachance 2012). We describe this new species based only on a single strain because of its ability to convert D-xylose to xylitol using sugarcane bagasse hemicellulose hydrolysate, a biotechnological trait of industrial interest. The strain CLQCA-24SC-025^T did not produce ascospores when grown on diluted V8 agar, Fowell acetate, cornmeal agar, and yeast carbon base agar supplemented with 0.01 % ammonium sulphate incubated at 15 and 25 °C until 28 days. This species was isolated from a sample of rotting wood, cultivated in YNB-D-xylose medium, and collected on Santa Cruz Island in the Galápagos Archipelago. The isolation of this novel species from rotting wood suggests that this substrate could be its ecological niche. *C. lachancei* can be distinguished from *C. galapagosensis* sp. nov. based on growth on cellobiose, L-rhamnose and DL-lactate, which are positive for the first species and negative for the novel species. However, sequencing is recommended to differentiate these species.

Description of *Cyberlindnera galapagoensis* sp. nov. Guamán-Burneo, Cadete, Portero, Carvajal Barriga and Rosa

In 5 % malt extract agar after 3 days at 25 °C, the cells are ovoid or occasionally elongated (1.9–2.9 × 2.6–10.5 µm), and occur singly or in pairs. Budding is multilateral (Fig. 2). A sediment and a ring are formed after a month in YM broth at 25 °C, but no pellicle was observed. On YM agar after 2 days at 25 °C, colonies are white-cream, smooth and glistening. In Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae are not present. Cultures of the individual strain grown on 5 % malt extract, Fowell acetate, cornmeal agar, diluted (1:9) V8 agar, 5 % malt extract agar and yeast carbon base agar supplemented with 0.01 % ammonium sulphate incubated at 15 and 25 °C until 21 days produce no ascospores. Fermentation of glucose, sucrose and raffinose is


Cyberlindnera xylosilytica UFMG-HMD-7.2 (JQ695900)
95
Cyberlindnera xylosilytica NRRL YB-2097T (EF550324)
56
Cyberlindnera xylosilytica LCF-118 NU1W51 (HM461705)
Candida maesa ATCC MYA-4698T (JQ812697)
61
Candida easanensis JCM 12477T (AY634571)
Cyberlindnera japonica NRRL YB-2750T (EF550323)
91
Candida adriatica ZIM 2334T (HE574661)
Candida hungchunana ATCC MYA-4701T (JQ812700)
76
Candida taoyuanica ATCC MYA-4700T (JQ812699)
Candida stauntonica ATCC MYA-4699T (JQ812698)
Candida pattaniensis JCM 12475T (AY634568)
98
Cyberlindnera amylophila NRRL YB-1287T (EF550319)
Cyberlindnera mississippiensis NRRL YB-1294T (EF550320)
Cyberlindnera veronae NRRL Y-7818T (EF550322)
53
Cyberlindnera fabianii NRRL Y-1871T (EF550321)
58
Candida maritima NRRL Y-17775T (EF550332)
Candida mycetangii NRRL Y-6843T (EF550330)
Candida nakhonratchasimensis JCM 12474T (AY634567)
Cyberlindnera americana NRRL Y-2156T (EF550328)
Cyberlindnera bimundalis NRRL Y-5343T (EF550329)
Cyberlindnera euphorbiae NRRL Y-17232T (EF550326)
80
Cyberlindnera meyerae NRRL Y-17236T (EF550327)
Cyberlindnera euphorbiiphila NRRL Y-12742T (EF550312)
Cyberlindnera samutprakarnensis JP52T (AB598079)
Candida mengyuniae JLHT (EU043158)
98
Cyberlindnera misumaiensis NRRL Y-7686T (EF550248)
Candida sp. NRRL Y-27103 (EF550307)
Cyberlindnera maclurae NRRL Y-5377T (EF550310)
Cyberlindnera petersonii NRRL YB-3808T (EF550311)
Cyberlindnera jadinii NRRL Y-1542T (EF550309)
Cyberlindnera xylebori NBRC 11048T (AB534167)
Cyberlindnera galapagoensis sp. nov. CLQCA-24SC-02T (KJ020281)
26
Candida sp. NRRL Y-27267 (EF550314)
99
Cyberlindnera lachancei NRRL Y-27008T (EF550313)
Candida takata ATCC MYA-4702T (JQ906764)
Candida vartiovaarae NRRL Y-670T (EF550315)
Cyberlindnera suaveolens NRRL Y-17391T (EU544674)
79
Cyberlindnera sargentensis NRRL YB-4139T (HM461618)
Cyberlindnera saturnus Y-17396T (EF550316)
90
Cyberlindnera mrakii NRRL Y-1364T (EF550317)
Cyberlindnera subsufficiens NRRL Y-1657T (EF550318)
Wickerhamomyces anomalus NRRL Y-366NT (EF550341)
99
Wickerhamomyces hampshirensis NRRL YB-4128T (EF550334)
0.05

◀**Fig. 1** Phylogenetic placement of *Cyberlindnera galapagoensis* sp. nov. among species of the *Cyberlindnera* clade based on a maximum likelihood analysis of the sequences of the D1/D2 domains of the large subunit rRNA gene, using the general time reversible model. *Bootstraps* were obtained from 100 iterations using 532 aligned nucleotide positions. *Bar*, number of nucleotide substitutions

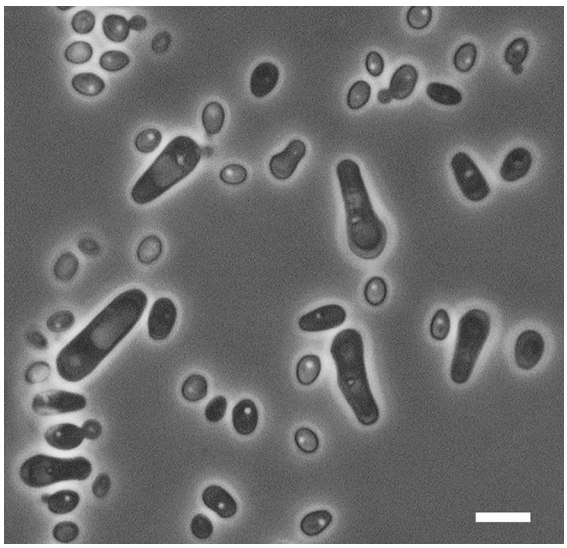


Fig. 2 Cells of *Cyberlindnera galapagoensis* sp. nov. on 5 % malt extract agar after 5 days at 25 °C. Bar, 5 μm

positive. Assimilation of carbon compounds: glucose, inulin, sucrose, raffinose, salicin, D-xylose, ethanol, glycerol, D-mannitol, D-glucitol, succinate (weak and slow), citrate (weak and slow), D-gluconate, xylitol and ethylacetate. No growth occurs on melibiose, galactose, lactose, trehalose, maltose, melizitose, soluble starch, cellobiose, L-sorbose, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, erythritol, ribitol, galactitol, *myo*-inositol, DL-lactate, *N*-acetyl-D-glucosamine, hexadecane, acetone and isopropanol. Assimilation of nitrogen compounds: positive for lysine and negative for nitrate and nitrite. Growth in amino-acid-free medium is positive. Growth at 37 °C is negative. Growth on YM agar with 10 % sodium chloride is positive (slow). Growth in 50 % glucose/yeast extract (0.5 %) is weak and slow. Acid production is positive. Starch-like compounds are not produced. In 100 μg cycloheximide mL^{-1}, growth is negative. Urease activity is negative. Diazonium Blue B reaction is negative.

Type

The type strain accession number of *Cyberlindnera galapagoensis* f.a., sp. nov., is CLQCA-24SC-025^T. It was isolated from rotting wood collected on Santa Cruz Island, Galápagos Archipelago, Ecuador cultured on YNB-D-xylose medium. It has been deposited in the Catholic University Yeasts Collection (CLQCA) in Ecuador as strain CLQCA-24SC-025^T, and is permanently preserved in a metabolically inactive state. Ex-type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 13997^T, and in the Collection of Microorganisms, DNA and Cells of Federal University of Minas Gerais (Coleção de Micro-organismos, DNA e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strain UFMG-CM-Y517^T. The Mycobank number is MB 812171.

Etymology

The epithet *galapagoensis* (ga.la.po.eńsis) L. nom. M. adj. *galapagoensis*, of or pertaining to the Galápagos Archipelago where the new species was isolated.

Acknowledgments This work was funded by the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq – Brazil, awarded to CAR), Fundação do Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG, awarded to CAR), Fundação do Amparo a Pesquisa do Estado de São Paulo (FAPESP, awarded to SSS), the Financiadora de Estudos e Projetos (FINEP, process number 2084/07, awarded to CAR) and the Pontificia Universidad Católica del Ecuador and SENESCYT (awarded to EJCB and CGB). We thank the Ecuadorian government for permission to collect in the Galápagos Archipelago. We thank Prof. Marc-André Lachance for providing the phylogenetic tree of the novel species.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Arrizon J, Mateos JC, Sandoval G, Aguilar B, Solis J, Aguilar MG (2012) Bioethanol and xylitol production from different lignocellulosic hydrolysates by sequential fermentation. J Food Process Eng 35:437–454

Arruda PV, Rodrigues RCLB, da Silva DDV, Felipe MGA (2011) Evaluation of hexose and pentose in pre-cultivation of *Candida guilliermondii* on the key enzymes for xylitol production in sugarcane hemicellulosic hydrolysate. Biodegradation 22:815–822

Barbosa MFS, Medeiros MB, Mancilha IM, Schneider H, Lee H (1988) Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii*. J Ind Microbiol 3:241–251

Bruinenberg PM, de Bot PHM, van Dijken JP, Scheffers WA (1983) The role of redox balances in the anaerobic fermentation of xylose by yeasts. Eur J Appl Microbiol Biotechnol 18:287–292

Cadete RM, Melo MA, Dussán KJ, Rodrigues RCLB, da Silva SS, Zilli JE, Vital MJS, Gomes FCO, Lachance MA, Rosa CA (2012) Diversity and physiological characterization of D-xylose-fermenting yeasts isolated from the Brazilian Amazonian Forest. PLoS One 7:e43135

Chen X, Jiang ZH, Chen S, Wensheng Q (2010) Microbial and bioconversion production of D-xylitol and its detection and application. Int J Biol Sci 6:834–844

Converti A, Perego P, Domínguez JM (1999) Xylitol production from hardwood hemicellulose hydrolysates by *Pachysolen tannophilus*, *Debaryomyces hansenii*, and *Candida guilliermondii*. Appl Biochem Biotechnol 82:141–151

da Silva SS, Afschar AS (1994) Microbial production of xylitol from D-xylose using *Candida tropicalis*. Bioprocess Eng 11:129–134

da Silva SS, Chandel AK (2012) D-xylitol: fermentative production, application and commercialization. Springer, Heidelberg

da Silva DDV, Felipe MGA (2006) Effect of glucose:xylose ratio on xylose redutase and xylitol dehydrogenase activities from *Candida guilliermondii* in sugarcane bagasse hydrolysate. J Chem Technol Biotechnol 81:1294–1300

da Silva SS, Vitolo M, Pessoa A Jr, Felipe MGA (1996) Xylose reductase and xylitol dehydrogenase activities of D-xylose-xylitol-fermenting *Candida guilliermondii*. J Basic Microbiol 36:187–191

de Barros Lopes M, Soden A, Henschke PA, Langridge P (1996) PCR differentiation of commercial yeast strains using intron splice site primers. Appl Environ Microbiol 62:4514–4520

de Mello MV (2009) Seleção de leveduras para bioconversão de D-xilose em xilitol. Master Dissertation, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo

Galápagos National Park, Ecuador (2012) Ministerio del Ambiente. Galápagos, 1st edn. http://www.galapagospark.org/nophprg.php?page=parque_nacional_introducidas_plantas_santa_cruz. Accessed 14 Sep 2012

Gárdonyi M, Österberg M, Rodrigues C, Spencer-Martins I, Hahn-Hägerdal B (2003) High capacity xylose transport in *Candida intermedia* PYCC 4715. FEMS Yeast Res 3:45–52

Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R (2010) Hemicelluloses for fuel ethanol: a review. Bioresour Technol 101:4775–4800

Guo C, Zhao C, He P, Lu D, Shen A, Jiang N (2006) Screening and characterization of yeasts for xylitol production. J Appl Microbiol 101:1096–1104

Kamat S, Khot M, Zinjarde S, Ravikumar A, Gade WN (2013) Coupled production of single cell oil as biodiesel feedstock, xylitol and xylanase from sugarcane bagasse in a biorefinery concept using fungi from the tropical mangrove wetlands. Bioresour Technol 135:246–253

Kurtzman CP, Fell JW, Boekhout T (2011) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam

Lachance MA (2012) In defense of yeast sexual life cycles: the forma asexualis—an informal proposal. Yeast Newslett 61:24–25

Lachance MA, Bowles JM, Starmer WT, Barker JSF (1999) *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. Can J Microbiol 45:172–177

Lachance MA, Boekhout T, Scorzetti G, Fell JW, Kurtzman CP (2011) *Candida* Berkhout (1923). In: Kurtzman CP, Fell J, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 987–1278

Leandro MJ, Gonçalves P, Spencer-Martins I (2006) Two glucose/xylose transporter genes from the yeast *Candida intermedia*: first molecular characterization of a yeast xylose-H^+ symporter. Biochem J 395:543–549

Leathers TD, Gupta SC (1997) Xylitol and riboflavin accumulation in xylose-grown cultures of *Pichia guilliermondii*. Appl Microbiol Biotechnol 47:58–61

Mohamad NL, Kamal NL, Mustapa SM, Mokhtar MN (2015) Xylitol biological production: a review of recent studies. Food Ver Int 31:74–89

Morais CG, Cadete RM, Uetanabaro AP, Rosa LH, Lachance MA, Rosa CA (2013a) D-xylose-fermenting and xylanase-producing yeast species from rotting wood of two Atlantic rainforest habitats in Brazil. Fungal Genet Biol 60:19–28

Morais CG, Lara CA, Marques S, Fonseca C, Lachance MA, Rosa CA (2013b) *Sugiyamaella xylanicola* sp. nov., a xylan-degrading yeast species isolated from rotting wood. Int J Syst Evol Microbiol 6:2356–2360

Mussatto SI, Roberto IC (2002) Xilitol: edulcorante com efeitos benéficos para a saúde humana. Braz J Pharm Sci 38:401–413

Neill DA, Jorgensen PM (1995) Catálogo de las plantas vasculares del Ecuador, St. Louis: Missouri Botanical Garden. http://www.mobot.org/mobot/research/ecuador/vegetationsp.shtml. Accessed 15 Dec 2012

Rao RS, Jyothi CP, Prakasham RS, Sarma PN, Rao LV (2006) Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*. Bioresour Technol 97:1974–1978

Rao RS, Bhadra B, Shivaji S (2008) Isolation and characterization of ethanol-producing yeasts from fruits and tree barks. Lett Appl Microbiol 47:19–24

Rosa CA, Lachance MA, Teixeira LCRS, Pimenta RS, Morais PB (2007) *Metschnikowia cerradonensis* sp. nov., a yeast species isolated from ephemeral flowers and their nitidulid beetles in Brazil. Int J Syst Evol Microbiol 57:161–165

Saha BC (2003) Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30:279–291

Sampaio FC, Chaves-Alves VM, Converti A, Passos FML, Coelho JLC (2008) Influence of cultivation conditions on xylose-to-xylitol bioconversion by a new isolate of *Debaryomyces hansenii*. Bioresour Technol 99:502–508

Schneder AS, Schettler A, Markowski A, Luettig B, Momma M, Seipt C, Hadem J, Wilhelmi M (2014) Assessment of xylitol serum levels during the course of parenteral nutrition including xylitol in intensive care patients: a case control study. Clin Nutr 33:483–488

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Winkelhausen E, Kuzmanova S (1998) Microbial conversion of D-xylose to xylitol. J Ferment Bioeng 86:1–14