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Candida sorbosivorans sp. nov., a new member of the genus *Candida* Berkhout

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A yeast, strain NCYC 2938^T, was isolated from contaminated industrial material. This material was involved in a cascade continuous process for oxidizing sorbitol (D-glucitol) to L-sorbose. The isolate is similar, although not identical, to *Candida geochares* and *Candida magnoliae* in its physiological characteristics. Sequence analysis of the 26S rDNA D1/D2 variable domain showed that it was similar to those of both *Candida* species, but differed sufficiently to be considered as a separate species. Both the physiological characteristics and the unique 26S rDNA D1/D2 sequence of NCYC 2938^T are described here, and the yeast has been named *Candida sorbosivorans* sp. nov. The type strain is NCYC 2938^T (= CBS 8768^T).

Keywords: *Candida sorbosivorans* sp. nov., new yeast species, 26S rDNA

INTRODUCTION

Using the identification methods of van der Walt & Yarrow (1984) and Yarrow (1998), a yeast, strain NCYC 2938^T, was found to be physiologically similar, but not identical, to *Candida geochares* and its close relative *Candida magnoliae* (Meyer *et al.*, 1998; Kurtzman & Robnett, 1998). Strain NCYC 2938^T was isolated from contaminated industrial material, involved in a cascade continuous process for oxidizing sorbitol (D-glucitol) to L-sorbose (used in producing vitamin C). The sequence of the 26S rDNA D1/D2 domain of NCYC 2938^T was determined and compared with those of *C. geochares* and *C. magnoliae* to establish whether it was an atypical strain of either yeast, or a different species of the genus *Candida* (see Kurtzman & Robnett, 1998). According to Kurtzman and his colleagues, conspecific yeast strains typically display fewer than 1% nucleotide substitutions in this region of the 26S rDNA, whereas strains belonging to separate species generally (though not always) display larger numbers of substitutions (Kurtzman, 1998; Kurtzman & Robnett, 1991, 1998; Peterson & Kurtzman, 1991). Our decision to describe NCYC 2938^T as a new species of the anamorphic genus *Candida*, namely *Candida sorbosivorans* sp. nov., is based on its physiological characteristics and the 26S rDNA sequence data.

The EMBL accession number for the 26S rDNA sequence of the D1/D2 region of NCYC 2938^T is AJ277846.

METHODS

Yeast strains and phenotypic characterization. The yeast strains examined in this study are listed in Table 1 and are available from the National Collection of Yeast Cultures (NCYC), Norwich, UK, and the Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands. All strains were grown on YM agar (0·3% yeast extract, 0·3% malt extract, 0·5% peptone, 1% glucose, 2% agar; pH 5·5) at 24 °C and maintained on YM agar slopes at 4 °C. The novel isolate was assigned the strain number NCYC 2938^T and was characterized phenotypically using the standard yeast-identification tests as described by van der Walt & Yarrow (1984) and Yarrow (1998).

Growth-rate comparisons for different carbon sources. Commercial D-glucitol (Sigma) was recrystallized twice from the pyridine–glucitol complex, which was thermally destroyed, and the pyridine was removed under reduced pressure (Strain, 1934, 1937). The D-glucitol was then recrystallized twice from methanol. All incubations were carried out at 25 °C in 10 ml medium in 100 ml conical flasks. The flasks were shaken reciprocally at approximately 75 cycles min^{-1} and 10 cm travel. Each yeast strain was grown overnight in 1% (w/v) Difco yeast extract with 50 mM substrate (D-glucose, D-glucitol or L-sorbose) in order to acclimatize (i.e. induce or derepress) the yeast, particularly to D-glucitol and L-sorbose. Each growth was then filtered through a membrane filter and resuspended in Difco yeast nitrogen base with 50 mM substrate corresponding to that on which it had been grown. The cell suspensions were adjusted initially to an $OD_{640} \approx 0{\cdot}3$. Each growth was measured at least four times at 640 nm and calibrated in terms of dry weight (Barnett, 1968). Doubling times were calculated from the linear relationship between the logarithm of dry weight and time, during exponential growth.

Table 1. Yeast strains examined in this study

For full species descriptions of *C. geochares* and *C. magnoliae*, refer to Meyer *et al.* (1998). Abbreviations: CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; NCYC, National Collection of Yeast Cultures, Norwich, UK.

Species	Strain	Source of isolate
Candida geochares	CBS 6870^T	Grassland soil, South Africa
Candida magnoliae	NCYC 742	Unknown
	NCYC 765	Unknown
	NCYC 2620^T	Flower of *Magnolia* sp.
Candida sorbosivorans	NCYC 2938^T	Sorbitol-to-sorbose continuous fermentation cascade

26S rDNA sequencing and sequence analysis. The variable D1 and D2 domains of the 26S rDNA were PCR-amplified directly from individual yeast colonies by using the protocol detailed by James *et al.* (1994) and the conserved fungal oligonucleotide primers NL1 and NL4 described by O'Donnell (1993). The amplified 26S rDNA PCR products were purified using a QIAGEN QIAquick PCR purification kit according to the manufacturer's instructions.

Direct sequencing of the purified 26S rDNA PCR products was performed using a *Taq* DyeDeoxy terminator cycle sequencing kit (PE Biosystems) and an Omnigene thermal cycler (Hybaid) according to the manufacturers' recommendations. The 26S rDNA sequences were determined using NL1 and NL4 as sequencing primers. Purified sequencing reaction mixtures were electrophoresed with a PE Biosystems model 373A automated DNA sequencer.

The determined 26S rDNA D1/D2 sequence of strain NCYC 2938^T was compared with sequences held in both the EMBL and GenBank sequence databases. A sequence alignment of the 26S rDNA sequences for NCYC 2938^T and its closest relatives was created by using the multiple-sequence alignment program PILEUP (Feng & Doolittle, 1987) contained within the GCG software package (Genetics Computer Group, 1991) version 8.1. Phylogenetic analyses were performed using PHYLIP (Felsenstein, 1993) version 3.572. A distance matrix was generated using the DNADIST program with the Jukes–Cantor distance measure and a rooted phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) and the NEIGHBOR program. The stability of individual branches of the tree was assessed by the bootstrap method (Felsenstein, 1985) with the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE. A total of 456 nucleotides was determined from the 26S rDNA D1/D2 region of NCYC 2938^T.

RESULTS AND DISCUSSION

Phenotypic characterization

The morphological and physiological characteristics of NCYC 2938^T were determined by using standard methods, as described by Yarrow (1998), and are shown in Table 2. Use of the yeast-identification program of Barnett (1996) revealed that strain NCYC 2938^T possessed physiological characteristics similar, though not identical, to those of *C. geochares* and its close relative *C. magnoliae* (see Kurtzman & Robnett, 1998). NCYC 2938^T differed from *C. geochares* by its ability to assimilate both raffinose (albeit slowly) and potassium nitrate and from *C. magnoliae* by its inability to assimilate either D-ribose or ethanol. As well as these assimilatory differences, NCYC 2938^T was found to grow at 40 °C. In contrast, strains of both *C. geochares* and *C. magnoliae* can grow only at temperatures up to 37 °C (and only weakly in the case of *C. geochares*) (Meyer *et al.*, 1998).

To assess the significance of the D-glucitol (sorbitol) growth characteristic of NCYC 2938^T, additional tests were performed. As this isolate had originally been recovered as a contaminant from a sorbitol-to-sorbose fermentation cascade (Table 1), the growth rates (measured as doubling times) for NCYC 2938^T and the type strains of *C. geochares* (CBS 6870^T) and *C. magnoliae* (NCYC 2620^T) grown on D-glucose, D-glucitol (sorbitol) or L-sorbose were measured. On D-glucose, the growth rates of *C. geochares* CBS 6870^T, *C. magnoliae* NCYC 2620^T and strain NCYC 2938^T were measured as $5{\cdot}8 \pm 0{\cdot}3$, $4{\cdot}1 \pm 0{\cdot}5$ and $4{\cdot}8 \pm 0{\cdot}3$ h, respectively. For D-glucitol (sorbitol), the rate of growth was determined only for NCYC 2938^T ($5{\cdot}6 \pm 0{\cdot}2$ h), as the other two yeasts were found to flocculate in the presence of this sugar. For L-sorbose, the growth rates of all three yeasts were extremely slow (doubling times in excess of 6·0 h) and no differences between the three yeasts were evident. These results indicated further clear physiological differences, consistent with the evolution of a new species adapted to growth in D-glucitol (sorbitol) and exhibiting an intermediate growth rate on D-glucose.

26S rDNA sequence analysis

The 26S rDNA D1/D2 variable domain of NCYC 2938^T was amplified *in vitro* by using the PCR and the nucleotide sequence was determined directly. This sequence was used initially to search the EMBL and GenBank sequence databases in an attempt to establish the identity of this yeast isolate. Results of the search revealed that the NCYC 2938^T D1/D2 sequence was most similar to those of *C. geochares* and its close relative *C. magnoliae* (Kurtzman & Robnett, 1998). However, the D1/D2 sequence of NCYC 2938^T

Table 2. Physiological characteristics of *C. sorbosivorans* sp. nov.

Characters are scored as: +, positive; L, delayed (latent); S, slow; W, weak; −, negative.

Character	Score	Character	Score
Fermentation of carbohydrates		**Assimilation of carbon compounds (cont.)**	
D-Glucose	+	Galactitol	−
Sucrose	+	D-Mannitol	+
Maltose	−	D-Glucitol	+
D-Galactose	−	Methyl α-D-glucoside	−
Lactose	−	Salicin	L
Cellobiose	−	DL-Lactate	−
α,α-Trehalose	−	Succinate	L
Melibiose	−	Citrate	+
Melezitose	−	Inositol	−
Raffinose	−	D-Glucono-1,5-lactone	+
Methyl α-D-glucoside	−	D-Glucosamine	−
Inulin	−	Methanol	−
Soluble starch	−	Ethanol	−
Assimilation of carbon compounds		Xylitol	+
D-Glucose	+	**Assimilation of nitrogen compounds**	
D-Galactose	S	Nitrate	+
L-Sorbose	+	Ethylamine	+
Sucrose	+	Cadaverine	+
Maltose	−	L-Lysine	+
Cellobiose	L	**Additional growth characteristics**	
α,α-Trehalose	−	10 % NaCl/5 % glucose	+
Lactose	−	15 % NaCl/5 % glucose	+
Melibiose	−	20 % NaCl/5 % glucose	+
Raffinose	L	50 % D-Glucose	+
Melezitose	W	60 % D-Glucose	+
Inulin	−	0·01 % Cycloheximide	S
Starch	−	0·1 % Cycloheximide	W
D-Xylose	−	Urease activity	−
L-Arabinose	−	1 % Acetic acid	−
D-Arabinose	−	Lipolytic activity	−
D-Ribose	−	Acid production	−
Glycerol	+	Arbutin hydrolysis	S
L-Rhamnose	−	Starch formation	−
Erythritol	S	Growth at 37 °C	+
Ribitol	+	Growth at 40 °C	+

differed by five base substitutions from that of the *C. geochares* type strain (CBS 6870^T) and by six base substitutions from that of the *C. magnoliae* type strain (NCYC 2620^T). This suggested that isolate NCYC 2938^T belonged to a separate species, as conspecific strains typically differ by no more than three base substitutions (i.e. < 1·0 % sequence divergence) in this region of the 26S rDNA (Kurtzman, 1998; Kurtzman & Blanz, 1998; Kurtzman & Robnett, 1998). Indeed, when the D1/D2 sequences of two other strains (NCYC 742 and NCYC 765) of *C. magnoliae* were determined, both were found to be identical to that of the type strain, NCYC 2620^T. In comparison, the D1/D2 sequences of the type strains of *C. geochares* (CBS 6870^T) and *C. magnoliae* (NCYC 2620^T) differ from one another by a total of nine base substitutions.

The 26S rDNA D1/D2 sequences of NCYC 2938^T, *C. geochares*, *C. magnoliae* and a number of other related *Candida* species (see Fig. 10 of Kurtzman & Robnett, 1998) were aligned and the derived distances were used to infer phylogenetic relationships. Fig. 1 shows a rooted tree (with *Candida blankii* as the outgroup) constructed by using the neighbour-joining method and depicts the phylogenetic placement of NCYC 2938^T in relation to *C. geochares*, *C. magnoliae* and some other *Candida* species. Although NCYC 2938^T is phylogenetically closely related to both *C. geochares* and *C. magnoliae*, with the three yeasts forming a distinct species group (bootstrap value 94 %), it would appear that NCYC 2938^T belongs to a hitherto unknown species of *Candida*. Therefore, on the basis of both the physiological and 26S rDNA sequence

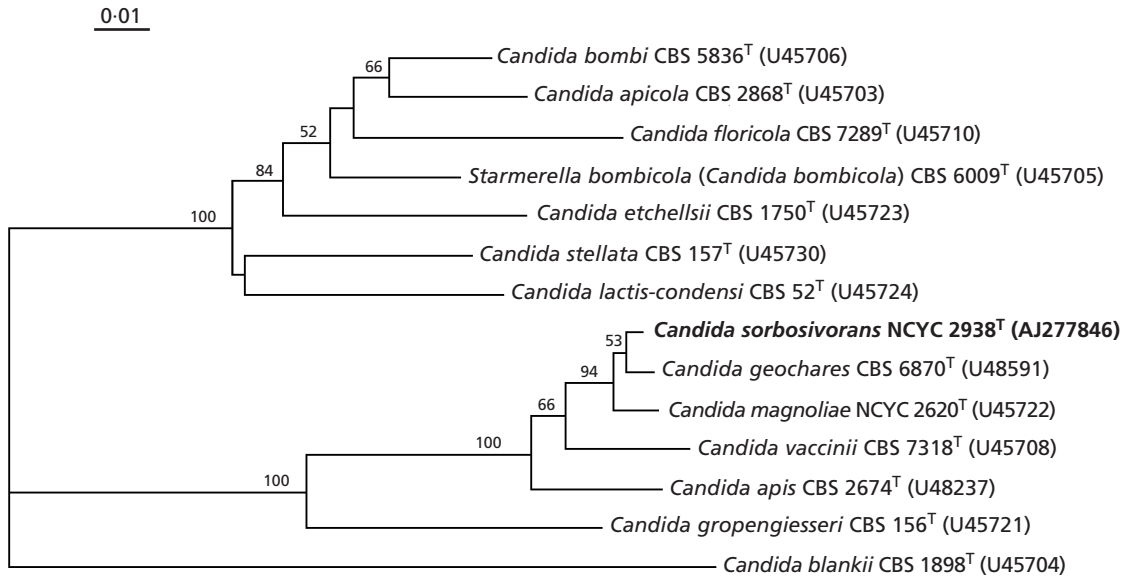

Fig. 1. Dendrogram showing the phylogenetic relationship between *Candida sorbosivorans* NCYC 2938^T and the type strains of other related *Candida* species, based on 26S rDNA D1/D2 gene sequences. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values, expressed as percentages of 200 replications, are given at branch nodes (only values $> 50\%$ are shown). Bar, 1 estimated base substitution per 100 nucleotide positions. The EMBL/GenBank accession numbers for the 26S rDNA D1/D2 gene sequences used in the analysis are shown in parentheses.

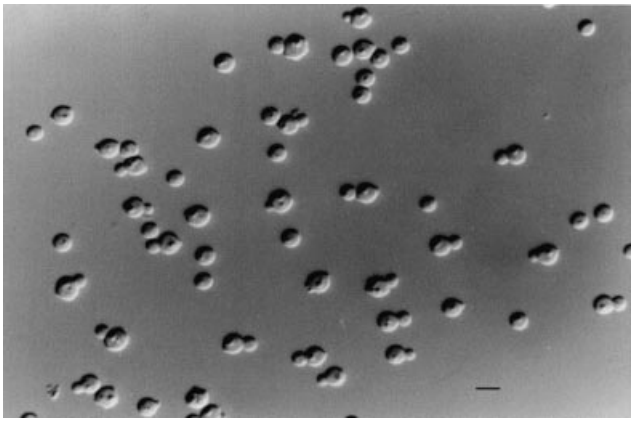

Fig. 2. Photomicrograph of strain NCYC 2938^T grown in YNBG medium (25 mM D-glucose) for 1 d at 25 °C. Bar, 5 μm.

data, we describe a new species, *C. sorbosivorans* sp. nov., to accommodate strain NCYC 2938^T.

Latin diagnosis of *Candida sorbosivorans* James, Bond *et* Roberts sp. nov.

Cultura in agaro morphologico (Difco) *post* 48 *horas ad* 24 °C: *cellulae ovoideae* (2·0–4·0 × 3·0–5·0 μm), *singulae*, *binae*, *adhaerentes*, *per gemmationem multipolarem reproducentes*. *Ascomata nulla post* 20 *dies* 24 °C *seu in agaro farina maydis confecto se* PDA *seu medio Gorodkowae*. *Glucosum et sucrosum fermentantur at non maltosum*, *galactosum*, *lactosum*, *cellobiosum*, *trehalosum*, *melibiosum*, *melezitosum*, *raffinosum*, *methyl* α-D-*glucosidum*, *inulinum*, *nec amylum*. *Glucosum*, *galactosum*, L-*sorbosum*, *sucrosum*, *cellobiosum*, *raffinosum*, *melezitosum*, *erythritolum*, *ribitolum*, D-*mannitolum*, D-*glucitolum*, *salicinum*, *acidum succinicum*, *acidum citricum*, *glucono*-D-*lactonum*, *glycerinum*, *xylitolum*, *nitrus kalicus*, *ethylaminum*, *lysinum*, *et cadaverinum assimilantur at non maltosum*, *trehalosum*, *lactosum*, *melibiosum*, *inulinum*, *amylum*, D-*xylosum*, L-*arabinosum*, D-*arabinosum*, D-*ribosum*, L-*rhamnosum*, *galactitolum*, *methyl* α-D-*glucosidum*, *acidum lacticum*, *inositolum*, D-*glucosaminum*, *metanolum nec alcohol aethylicum*. *Crescit in medio cum* 60 % *glucoso et in medio* 0·1 % *cycloheximido addito*. *Non crescit in medio* 1 % *acido acetico addito*. *Typus depositus in collectionis* 'National Collection of Yeast Cultures', Norwich, *Britannia* (NCYC 2938^T).

Description of *Candida sorbosivorans* James, Bond *et* Roberts sp. nov.

Candida sorbosivorans (sor.bos.i.vor′ans. N.L. *sorbosum* sorbose; L. part. adj. *vorans* devouring; N.L. gen. n. *sorbosivorans* devouring sorbose, referring to the ability of the new species to assimilate sorbose).

On morphology agar, after 48 h at 24 °C, the cells are spherical to ovoid (2·0–4·0 × 3·0–5·0 μm) and occur singly, in pairs or in groups (Fig. 2). Budding is multilateral. No ascosporulation was observed after cultures had been incubated for 3 weeks at 24 °C on either corn-meal agar, potato-dextrose agar or Gorodkowa agar. For a summary of the physiological

and other growth characteristics of strain NCYC 2938^T, see Table 2. Cultures of the type strain have been deposited with the National Collection of Yeast Cultures, Norwich, UK (NCYC 2938^T), and with the Centraalbureau voor Schimmelcultures, Delft, The Netherlands (CBS 8768^T).

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