***Zygosaccharomyces lentus* sp. nov., a new member of the yeast genus *Zygosaccharomyces* Barker**

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Unusual growth characteristics of a spoilage yeast, originally isolated from spoiled whole-orange drink and previously identified as *Zygosaccharomyces bailii*, prompted careful re-examination of its taxonomic position. Small-subunit rRNA gene sequences were determined for this strain and for four other strains also originally described as *Z. bailii* but which, in contrast to other strains of this species, grew poorly or not at all under aerobic conditions with agitation, failed to grow in the presence of 1% acetic acid and failed to grow at 30 °C. Comparative sequence analysis revealed that these strains represented a phylogenetically distinct taxon closely related to, but distinct from, *Z. bailii* and *Zygosaccharomyces bisporus*. Furthermore, sequence analysis of the internal transcribed spacer (ITS) region showed that, while all five strains had identical ITS2 sequences, they could be subdivided into two groups based on ITS1 sequences. Despite such minor inter-strain sequence variation, these yeasts could readily be distinguished from all other currently described *Zygosaccharomyces* species by using ITS sequences. On the basis of the phylogenetic results presented, a new species comprising the five strains, *Zygosaccharomyces lentus* sp. nov., is described and supporting physiological data are discussed, including a demonstration that growth of this species is particularly sensitive to the presence of oxygen. The type strain of *Z. lentus* is NCYC D2627^T.

Keywords: yeast, *Zygosaccharomyces lentus*, preservative resistance, spoilage

INTRODUCTION

Zygosaccharomyces is a yeast genus often regarded as synonymous with food spoilage. General characteristics of *Zygosaccharomyces* yeasts are fermentation of sugars, osmotolerance, resistance to preservatives including sulphite, sorbic acid and ethanol, formation of heat-resistant ascospores and fructophily (preference for fructose). *Zygosaccharomyces* includes some of the most osmotolerant organisms known, yeasts able to resist concentrations of food preservatives vastly in

excess of those normally, or legally, encountered. Foods particularly at risk are acidic, pH 2.5–5.0, containing high concentrations of fermentable sugars (Tilbury, 1980; Thomas & Davenport, 1985). These include fruit juices, soft drinks, juice concentrates and sugar syrups, candied fruit, jams and preserves, cream eggs, honey, tomato sauce and wines. Spoilage results in yeast clouds, particulates, taints and off-flavours, odours and excessive gas production. Yeast fermentation can generate CO₂ up to 6 bar over-pressure, capable of distorting packaging, causing bottles to explode and rupturing cans and kegs. Though the *Zygosaccharomyces* are not human pathogens, product spoilage by these yeasts can result in serious injury, particularly to the eye, caused by exploding glass bottles (Grinbaum *et al.*, 1994).

The genus *Zygosaccharomyces* was introduced in 1901 by Barker (van der Walt & Johannsen, 1975) for yeast

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Abbreviation: ITS, internal transcribed spacer.

The EMBL accession numbers for the sequences reported in this paper are Y16814 (18S rDNA of NCYC D2627^T), Y16815 (ITS1 region of NCYC D2627^T), Y16816 (ITS2 region of NCYC D2627^T) and Y16817 (ITS1 region of NCYC 2406).

species in which asci resulted from the conjugation of two individual cells and in which the ascospores were smooth. The genus *Zygosaccharomyces* is closely related to that of the brewing/baking yeasts, *Saccharomyces*. Indeed, for many years *Zygosaccharomyces* yeasts were classified under *Saccharomyces* and misnomers such as *Saccharomyces rouxii* still persist in the literature. Recent authorities (Yarrow, 1984; Barnett *et al.*, 1990) have reinstated *Zygosaccharomyces* as a separate genus, though small-subunit rRNA sequencing has since revealed that these yeasts are phylogenetically intermixed with species of the genera *Kluyveromyces*, *Saccharomyces* and *Torulaspora* (James *et al.*, 1994, 1996; Cai *et al.*, 1996). However, despite this phylogenetic heterogeneity, results have shown that *Zygosaccharomyces bailii*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces mellis* and *Zygosaccharomyces rouxii*, the four most noted spoilage species, are not only closely related to one another, but also form a distinct (bootstrap value, 100%) genealogical lineage (James *et al.*, 1997) worthy of separate generic status.

Z. bailii is a yeast renowned for its exceptional resistance to preservatives. Ingram (1960) reported growth in 500 p.p.m. benzoic acid at pH < 3.0. Similar resistance to sorbic acid and sulphite has been recorded (Pitt, 1974; Hammond & Carr, 1976; Neves *et al.*, 1994). Growth in preservatives causes adaptation of *Z. bailii*, the acquired tolerance of extreme levels of preservatives, possibly as a result of an induced preservative pump (Warth, 1977, 1989). *Z. bailii* is also remarkable in that it has been reported to cause spoilage from an inoculum of one cell per litre or per package (Pitt & Hocking, 1985; Davenport, 1996). *Z. bailii* is osmotolerant, growing well in fruit-juice concentrates, and its natural habitat may be in shrivelled, mummified fruits in orchards (Davenport, 1975). Genealogically, *Z. bisporus* is the closest relative of *Z. bailii* (James *et al.*, 1994) and as a result shares many of its characteristics. However, *Z. bisporus* is marginally more osmotolerant and appears less resistant to preservatives (Tilbury, 1980) and *Z. bisporus* is unable to grow on sucrose (Barnett *et al.*, 1990), whereas *Z. bailii* uses this substrate, albeit poorly. *Z. rouxii* is also renowned for its extreme osmotolerance, being found at a water activity of 0.62 in fructose and 0.65 in glucose/glycerol (Tilbury, 1980). As a consequence, *Z. rouxii* is particularly noted for its spoilage of sugar syrups and juice concentrates. This yeast cannot ferment sucrose but, like *Z. bailii*, displays a high degree of resistance to preservatives. In contrast, *Z. mellis* has less frequently been reported to cause spoilage (Sand & van Grimsven, 1976), though this is perhaps due more to the fact that, until recently, this yeast was regarded as a synonym of *Z. rouxii* (Barnett *et al.*, 1990). Indeed, it was only through a nuclear (n) DNA–nDNA hybridization study of the *Zygosaccharomyces* that Kurtzman (1990) established this yeast as a genetically distinct species, a finding later confirmed by small-subunit rRNA sequencing (James *et al.*,

1994). *Z. mellis*, like *Z. rouxii*, is very osmotolerant and is frequently isolated from honey (Munitis *et al.*, 1976; Farris *et al.*, 1981). *Z. mellis* is also unable to use sucrose as a substrate, unless this sugar has been chemically inverted by low pH (Scarr & Rose, 1966).

Here *Zygosaccharomyces lentus* sp. nov., a new species of *Zygosaccharomyces* that is heavily implicated in the spoilage of foods is reported. It is genealogically closely related to *Z. bailii*, *Z. bisporus*, *Z. rouxii* and *Z. mellis* but is physiologically distinct, and it appears probable that strains of this new species have previously been misidentified as *Z. bailii*.

METHODS

Yeast strains. 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 Portuguese Yeast Culture Collection (IGC), Oeiras, Portugal. *Z. bisporus* strain BV7 was obtained from Unilever Research. *Saccharomyces cerevisiae* X1280-1B is a commonly used haploid strain, widely regarded as a typical wild-type, and was obtained from the Yeast Genetic Stock Center (Berkeley, CA, USA). 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 agar slopes at 4 °C.

Growth conditions

Starter cultures were grown in 10 ml YEPD broth (1% yeast extract, 2% bacteriological peptone, 2% glucose; pH 4.0) in 30 ml capped MacCartney bottles for 48 h at 26 °C. Experimental cultures were inoculated with 1 mg dry cells l⁻¹ (2 × 10⁴ cells ml⁻¹). Shaking cultures comprised 50 ml YEPD in 125 ml conical flasks, shaken on an Infors orbital shaker, 25 mm radius, at 140 r.p.m. at 26 °C. Static cultures comprised either 50 ml cultures in conical flasks or 10 ml cultures in capped 30 ml MacCartney bottles, incubated without movement.

Modified atmospheres. Where indicated, cultures were grown (shaken or static) in atmospheres of air, nitrogen or oxygen. This was achieved by placing inoculated flasks in gas jars, normally used for anaerobic plate incubation. Nitrogen or oxygen was flushed through the jars for 2 min before they were sealed. Jars containing flasks were then placed on orbital shakers or incubated without movement.

Growth in acetic acid. Ability of yeast strains to grow on media containing 1% acetic acid was determined by using MAC agar plus 1% acetic acid (1% mannitol, 1% yeast extract, 2% agar and glacial acetic acid, added after autoclaving, at 1%, v/v). Growth on this medium is a characteristic of *Z. bailii* (Davenport, 1981).

Phenotypic characterization. Yeast strains were characterized phenotypically by using the API ID 32C strip system (bioMérieux) for quick identification and standard methods for complete yeast identification (van der Walt & Yarrow, 1984).

PCR amplification of the 18S rDNA and internal transcribed spacer (ITS) region. Amplification of the 18S rRNA gene was performed as described by James *et al.* (1994). The entire ITS region was amplified with primers P3490 and pITS4 as previously described by James *et al.* (1998). The amplified products were purified by using a QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions.

Table 1. Yeast strains used in this study

Species	Strain	Source of isolate
<i>Saccharomyces cerevisiae</i>	X2180-1B	Unknown
<i>Zygosaccharomyces bailii</i>	NCYC 417	Unknown
	NCYC 563	Sorghum brandy
	NCYC 1416 ^T	Unknown
	NCYC 1427	Unknown
	NCYC 1766	Spoiled grape and blackcurrant juice
<i>Zygosaccharomyces bisporus</i>	BV7	Middle-Eastern soft drink spoilage
	NCYC 1495 ^T	Unknown
	IGC 5207	Spoiled orange beverage, UK
<i>Zygosaccharomyces lentus</i>	IGC 5316	Wine, France
	NCYC 1601	Orange squash drink
	NCYC 2406	Spoiled tomato ketchup, UK
	NCYC D2627 ^T	Spoiled whole-orange juice, UK

Sequence determination and analysis. Direct sequencing of both the 18S rRNA gene and ITS PCR products was performed by using a *Taq* DyeDeoxy terminator cycle-sequencing kit (ABI) and an Omnigene thermal cycler (Hybaid) according to the manufacturers' recommendations. Complete 18S rDNA and ITS sequences were determined by using primers described previously by James *et al.* (1994, 1996), with the exception that primers WIL1 and WIL2 (James *et al.*, 1998) were used for 18S rDNA sequencing in preference to primers M760 and P760 (James *et al.*, 1994), as they generated higher quality template for subsequent sequence analysis. Purified sequence reaction mixtures were electrophoresed with an Applied Biosystems model 373A automatic DNA sequencer.

Analysis of sequence data. The 18S rRNA gene sequences were aligned by using the multiple-sequence alignment program PILEUP (Feng & Doolittle, 1987) contained within the GCG software package (Genetics Computer Group, 1991) version 8.1. Alignments were adjusted manually. 18S rRNA gene sequence identity values were calculated by using the program GAP. Phylogenetic analyses were performed by using PHYLIP (phylogeny inference package; Felsenstein, 1993) version 3.572. A distance matrix was obtained by using the DNADIST program and an unrooted phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) and the NEIGHBOR program. The stability of the individual branches of the tree was assessed by using the bootstrap method (Felsenstein, 1985) with the SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs. The ITS1 and ITS2 sequences for the five strains of the novel *Zygosaccharomyces* species were aligned and compared with those of *Z. bailii* and *Z. bisporus*. The resulting alignments were adjusted manually.

RESULTS AND DISCUSSION

Strain NCYC D2627^T was originally isolated in the UK in the mid-1980s from a spoiled whole-orange drink. It was provisionally identified as a strain of *Z. bailii* on the basis of utilization of carbohydrates as growth substrate, as demonstrated by the API ID 32C strip system. Furthermore, growth of this strain on malt extract or on cornmeal agar at 24 °C for 1–2 weeks resulted in the production of distinctive con-

Fig. 1. Photomicrograph of NCYC D2627^T grown on malt extract agar for 10 d at 22 °C. Many cells have formed distinctive conjugation tubes, while other cells contain two to four smooth ascospores. Bar, 10 µm.

jugation tubes (referred to by some authors as 'schmoos': Lemontt & MacKay, 1977; Drubin, 1991) and subsequent formation of one to four smooth ascospores (Fig. 1). This morphology is also typical of *Z. bailii* (Thomas & Davenport, 1985).

Fig. 2. Lack of growth of NCYC D2627^T, NCYC 1601, NCYC 2406, IGC 5207 and IGC 5316 in shaking culture at 140 r.p.m. on a 25 mm orbital radius shaker, measured after 2 d at 26 °C in YEPD medium. Other strains shown are *Z. bailii* NCYC 563 and NCYC 1766, *S. cerevisiae* X2180-1B and *Z. bisporus* BV7.

Fig. 4. Growth of NCYC D2627^T in YEPD medium under modified atmospheres. Cultures (50 ml in 125 ml conical flasks) were placed in gas jars, which were then flushed with nitrogen, oxygen or air for 2 min. Jars were then sealed and incubated with shaking (140 r.p.m., 25 mm orbital radius; open bars) or without (static; filled bars). Growth was measured after 7 d at 26 °C.

Fig. 3. Growth of (a) *Z. bailii* NCYC 563 and (b) NCYC D2627^T in 50 ml YEPD medium in a 125 ml conical flask at 26 °C. Growth in shaking (■) (140 r.p.m., 25 mm orbital radius) and static (●) cultures is shown. Growth was measured as OD₆₀₀ and converted to dry weight by using a calibration graph.

Growth in shaking and static cultures

It was observed that, when cultured, strain NCYC D2627^T required a much longer period than *Z. bailii* to reach a visible level of growth and appeared to have a

much slower growth rate. A further anomaly was observed when cultures were incubated in shaking conical flasks. Growth of typical *Z. bailii* strains was enhanced by shaking, whereas no growth was observed with shaken cultures of strain NCYC D2627^T (Fig. 2). *Z. bisporus* and *S. cerevisiae* also grew well in shaking cultures (Fig. 2), as did *Z. rouxii* (data not shown). Growth characteristics of both static and shaking cultures in YEPD broth at pH 4.0 were investigated in greater detail, as shown in Fig. 3. Growth of *Z. bailii* NCYC 563 was shown to be significantly better in shaking culture than in static culture. Growth of *Z. bailii* was complete and reached stationary phase within 90 h, but slower growth, with a doubling time of 4 h, was observed in the static culture, compared to 2.5 h when the culture was shaken. Final yield (Fig. 3a) was also considerably greater in the shaking culture, with three times more cells being produced compared to the static culture.

Fig. 3(b) demonstrates a diametrically opposite response to agitation in cultures of NCYC D2627^T. No growth was obtained with shaking, even after 200 h incubation. Growth in static culture was steady but slow, with the doubling time estimated to be 15 h. The final yield was also low compared to *Z. bailii* NCYC 563, at only 380 mg dry weight cells l⁻¹.

Growth in modified atmospheres

The lack of growth of NCYC D2627^T in shaking culture was investigated briefly. Growth inhibition was likely to be due either to a greater sensitivity of this yeast to oxygen or to the physical agitation of shaking. This was examined by growing cells in both shaking and static conditions under an atmosphere of nitrogen, oxygen or air (Fig. 4). In shaking cultures, growth was only obtained in flasks under an atmosphere of

Fig. 5. Dendrogram showing the phylogenetic relationship of *Z. lentus* sp. nov. to type strains of other species of the genera *Kluyveromyces*, *Candida*, *Saccharomyces*, *Torulaspora* and *Zygosaccharomyces*, based on 18S rRNA 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 points (only values >50% are shown). Bar, one estimated base substitution per 1000 nucleotide positions.

nitrogen, with no growth observed under air or oxygen. Static culture resulted in growth under air or nitrogen but, again, not in oxygen. Growth of NCYC D2627^T in shaking culture under nitrogen demonstrates that the physical agitation of shaking did not affect growth. Inhibition of growth of NCYC D2627^T in static culture under oxygen shows this strain to be sensitive to oxygen. The higher levels of oxygen available in a shaking culture are therefore likely to inhibit growth. However, results suggest that a minimum level of oxygen is required for optimal growth, as the final yield of cells in a static culture was higher in air than in nitrogen. Lack of growth in shaking culture was also observed in a further four strains supposedly belonging to *Z. bailii*, NCYC 1601, NCYC 2406, IGC 5207 and IGC 5316, all derived from spoiled fruit juice-derived foods.

Further work showed that the lack of growth of strains NCYC 1601, NCYC 2406, NCYC D2627^T, IGC 5207

and IGC 5316 in shaking culture only occurred at temperatures close to the upper limit for growth and that these strains grew well in shaking culture at temperatures of <24 °C (data not shown). Therefore, these strains cannot be regarded as microaerophiles. We speculate that these strains are sensitive to oxidative damage when stressed by temperatures close to the upper growth limit.

Growth in 1% acetic acid

Z. bailii is a preservative-resistant yeast able to grow in the presence of 1% acetic acid, a fact used as a diagnostic test for this species (Barnett *et al.*, 1990). Growth tests on MAC agar plus 1% acetic acid showed growth of *Z. bailii* strains NCYC 563 and NCYC 1766 and *Z. bispurus* BV7 but not of any of the five strains unable to grow in shaking culture, NCYC D2627^T, NCYC 1601, NCYC 2406, IGC 5207 and

Fig. 6. Aligned ITS1 sequences of strains NCYC D2627^T (2627) and NCYC 2406 (2406), *Z. bailii* strains NCYC 417 (417), NCYC 1416^T (1416) and NCYC 1427 (1427), and *Z. bisporus* strain NCYC 1495^T (1495). Dots indicate identity, dashes indicate gaps introduced to maximize alignment.

IGC 5316. This shows not only that these yeasts are more sensitive to acetic acid but that they are readily distinguished from *Z. bailii* strains by this test.

Maximum growth temperature

A further physiological distinction between the five strains and *Z. bailii* concerned the temperature range for growth. *Z. bailii* is reported to be able to grow at 30 °C, with strain-dependent growth at 35 and 37 °C (Barnett *et al.*, 1990). However, strains NCYC D2627^T, NCYC 1601, NCYC 2406, IGC 5207 and IGC 5316 did not grow, even in static culture, at 30 °C or above.

Slow growth rate, lack of growth in shaking culture, inhibition of growth by 1 % acetic acid and inability to grow at 30 °C all suggested that the taxonomic status of these five strains should be re-examined by using 18S rRNA gene sequencing, as previously applied to

Zygosaccharomyces, *Torulaspora* and *Saccharomyces* species (James *et al.*, 1994, 1996, 1997).

18S rRNA gene sequence analysis

The 18S rRNA gene sequences of strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T were amplified *in vitro* by PCR and their nucleotide sequences were determined directly. These five newly determined sequences were aligned with complete or near-complete 18S rRNA gene sequences of all species of the genus *Zygosaccharomyces*, as well as representative members of the genera *Kluyveromyces*, *Saccharomyces* and *Torulaspora*. Levels of sequence identity were calculated (data not shown) and the distances derived were used to infer phylogenetic relationships. Fig. 5 shows an unrooted tree constructed by using the neighbour-joining method and depicts the phylogenetic relationship between strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T and other members of the genus *Zygosaccharomyces* and close relatives. Comparative analysis of the 18S rRNA gene sequence data revealed that strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T had identical sequences, which displayed high levels of sequence identity with the other *Zygosaccharomyces* species (ranging from 98.1 to 99.8 %). However, despite such values, the 18S rRNA gene sequences of these strains were not identical to the *Z. bailii* type strain (NCYC 1416^T), exhibiting five base differences. As Fig. 5 shows, these strains are closely related phylogenetically to both *Z. bailii* and *Z. bisporus* but are clearly representatives of a distinct *Zygosaccharomyces* species. Indeed, a detailed analysis of the sequences revealed that strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T could be distinguished readily from *Z. bailii*, its genealogically close relative *Z. bisporus* (James *et al.*, 1994) and all other *Zygosaccharomyces* species by the possession of a distinctive seven-base signature sequence (TTTT-ACT) at positions 678–682 (*S. cerevisiae* numbering; Mankin *et al.*, 1986) in the V4 region of the gene (Neefs *et al.*, 1991). In comparison, *Z. bailii* has an eight-base sequence (TTTTAATT), while *Z. bisporus* has a nine-base sequence (TTTTAATTT), at these positions (James *et al.*, 1994).

ITS sequence analysis

The nucleotide sequences of the ITS1 and ITS2 region for all five strains of the novel *Zygosaccharomyces* species were determined by sequencing PCR-amplified fragments directly. Unlike *Z. bailii*, where size variation in both ITS1 and ITS2 was found between different strains (James *et al.*, 1996), no such size polymorphisms were observed between strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T. The ITS1 and ITS2 regions were 255 and 227 bp, respectively, and as such are smaller than their counterparts in both *Z. bailii* (ITS1, ranging from 266

Fig. 7. Aligned ITS2 sequences of NCYC D2627^T (2627), *Z. bailii* strains NCYC 417 (417), NCYC 1416^T (1416) and NCYC 1427 (1427), and *Z. bisporus* strain NCYC 1495^T (1495). Dots indicate identity, dashes indicate gaps introduced to maximize alignment.

to 281 bp; ITS2, ranging from 258 to 278 bp) and *Z. bisporus* (ITS1, 269 bp; ITS2, 251 bp) strains (James *et al.*, 1996).

The ITS1 and ITS2 sequences of the five strains of the novel *Zygosaccharomyces* species were aligned with those of *Z. bailii* strains NCYC 417, NCYC 1416^T and NCYC 1427 and *Z. bisporus* strain NCYC 1495^T and these alignments are shown in Figs 6 (ITS1) and 7 (ITS2). As Fig. 6 shows, the five strains of the novel *Zygosaccharomyces* species could be subdivided into two groups, based on a single base substitution (C to A) in their ITS1 sequences. The first group comprised strains IGC 5207, IGC 5316, NCYC 1601 and NCYC D2627^T, while the second group comprised strain NCYC 2406 only. In contrast, all five strains possess identical ITS2 sequences (Fig. 7).

As Figs 6 and 7 show, the five strains can be distinguished readily from *Z. bailii* and *Z. bisporus* strains on the basis of their distinctive ITS1 and ITS2 sequences. To date, three different ITS1 sequence variants have been identified from *Z. bailii* strains (James *et al.*, 1996). However, it is quite clear from the

sequence data presented that strains IGC 5207, IGC 5316, NCYC 1601, NCYC 2406 and NCYC D2627^T have been incorrectly identified as *Z. bailii* strains. The ITS1 and ITS2 sequence data are in good agreement with both the 18S rRNA gene sequence and the physiological data (Fig. 5) and clearly identify these five strains as belonging to a hitherto unknown species of the genus *Zygosaccharomyces*.

Latin diagnosis of *Zygosaccharomyces lentus* sp. nov.

Cultura in agar 'morphologica' (Difco) *post* 48 horas *ad* 24 °C: *cellulae globosae vel ovoideae* (3.0–8.0 × 3.0–11.0 µm), *singulae vel binae, adhaerentes, per gemmationem multipolarem reproducentes. In agar farina Zeae maydis vel tuberibus Solani tuberosi concocto pseudomycelium primitivum adest. Asci ovoidei per conjugationem cellularum vegetativarum oriuntur, ascosporas ovales continentes, ex ascis non liberantur. Post* 7–14 *dies ad* 24 °C *in agar farinae Zeae maydis formantur. Glucosum et sucrosum fermentantur at non galactosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitose, inulinum nec amyllum. Glucosum, alcohol aethylicum, glycerinum, D-glucitolum, glucono-D-lactonum, ethylaminum et cadaverinum assimilantur at non galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitose, inulinum, amyllum, xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, erythritolum, ribitolum, galactitolum, D-mannitolum, methylum α-D-glucosidum, salicinum, acidum lacticum, acidum succinicum, acidum citricum, inositolum, D-glucosaminum, metanolum, xylitolum nec nitras kalicus. Crescit in medio cum* 50 % *glucoso. Non crescit in medio* 0.01 % *cycloheximido addito, neque in medio* 1 % *acido acetico addito, neque in medio vitaminis egente. Typus depositus in collectionis* 'National Collection of Yeast Cultures', Norwich, Britannia (NCYC D2627^T).

Description of *Zygosaccharomyces lentus* sp. nov.

Zygosaccharomyces lentus (len'tus. L. adj. *lentus* slow, apathetic, referring to the slow growth of this yeast).

On morphology agar, after 48 h growth at 24 °C, the cells are spherical to ovoid (3.0–8.0 × 3.0–11.0 µm) and occur singly, in pairs or in groups. Budding is multipolar. Ill-formed pseudohyphae are observed in cultures grown on corn meal agar and potato agar. Oval asci containing two to four round-to-oval ascospores are formed after incubation for 1 to 2 weeks at 24 °C on corn meal agar (Fig. 1). Asci are persistent. Conjugation occurs between separate cells. Ferments glucose and sucrose. Does not ferment galactose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin or starch. Assimilates glucose, ethanol, glycerol, D-glucitol, D-glucono-1,5-lactone, ethylamine hydrogen chloride and cadaverine. Does not assimilate galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raf-

finose, melezitose, inulin, starch, xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, ribitol, galactitol, D-mannitol, methyl α -D-glucoside, salicin, DL-lactate, succinate, citrate, inositol, D-glucosamine, methanol, xylitol or potassium nitrate. Growth occurs in the presence of 50% glucose. No growth occurs in the presence of 0.01% cycloheximide or 1% acetic acid or in vitamin-free medium. Cultures of the type strain, NCYC D2627^T, have been deposited in the National Collection of Yeast Cultures, Norwich, UK.

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