International Journal of Systematic and Evolutionary Microbiology (2002), **52**, 1369–1375 DOI: 10.1099/ijs.0.02088-0

Candida davenportii sp. nov., a potential soft-drinks spoilage yeast isolated from a wasp

M. Stratford,[1] C. J. Bond,[2] S. A. James,[2] I. N. Roberts[2] and H. Steels[1]

[1] Microbiology Section, Unilever R&D, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK

[2] National Collection of Yeast Cultures, Food Safety Science Division, Institute of Food Research, Colney Lane, Norwich NR4 7UA, UK

Author for correspondence: Malcolm Stratford. Tel: +44 1234 222 670. Fax: +44 1234 222 777. e-mail: malcolm.stratford@unilever.com

During a survey of yeast ecology in a soft-drinks production facility, a dead wasp was removed from the sampling tap of an external sugar-syrup storage tank. A yeast isolated from the dead wasp was found to be similar, although not identical, in its physiological characteristics to *Candida lactis-condensi* and *Candida stellata*. Sequence analysis of the 26S rDNA D1/D2 variable domain revealed that this isolate was most closely related to *C. stellata*, but differed sufficiently in its D1/D2 sequence to indicate that it belonged to a separate species. The yeast species has been named *Candida davenportii* sp. nov.; the type strain is NCYC 3013^T (= CBS 9069^T). *C. davenportii* sp. nov. was osmotolerant, moderately preservative-resistant and able to grow in very acidic conditions, i.e. pH 1·4. This yeast grew well in fruit-containing soft drinks, cola-type beverages and a synthetic soft drink and is therefore a potential cause of spoilage of soft drinks and other sugary food products. Other related yeast species in the same taxonomic clade as *C. davenportii* sp. nov. are also osmotolerant, growing in <50% (w/v) sugar. Many of these species are associated with insects, specifically bees, bumblebees and leafcutter bees, and many have been reported as the causative agent of spoilage of sugary foods, such as condensed milk, fruit juices and concentrates. It is proposed that *C. davenportii* sp. nov. and other closely related yeasts are primarily associated with Aculeates (bees and wasps). In turn, bees and wasps are attracted by sugary residues in foods such as fruit juices and concentrates, forming the source of infection of these yeasts and thus instigating spoilage.

Keywords: *Candida stellata*, *Candida lactis-condensi*, insects, osmotolerance, preservative resistance

INTRODUCTION

Due to their low pH, soft drinks constitute a hostile environment in which the great majority of microbes die, although *Escherichia coli* O157 and *Salmonella* species can persist for weeks in chilled, fruit juices (Goverd *et al*., 1979; Zhao *et al*., 1993). Spoilage of soft drinks is caused by a limited number of yeasts, moulds and acid-tolerant bacteria. Spoilage effects include formation of clouds, particulates, taints and excessive gas (Stratford *et al*., 2000). Infection of soft drinks commonly occurs via raw materials, returned bottles or aerial vectors (Sand, 1971; Tilbury, 1980; Byrne, 1994). Insects are increasingly recognized as a vector for yeasts. Many insects carry yeasts and insect frass, notably from fruit flies (*Drosophila* sp.), is particularly rich in soft-drinks spoilage yeasts (Lachance *et al*., 1995; Barnett *et al*., 2000).

Although many of the 800 or so yeasts discovered hitherto have been found in soft drinks or fruit juices (Barnett *et al*., 2000), relatively few species can grow in this environment or cause spoilage (Pitt & Hocking, 1997). Soft-drinks factories similarly contain large numbers of yeast isolates and micro-organisms of great taxonomic diversity (Sand, 1970, 1973; Sand & van Grinsven, 1976a, b). In a 'forensic approach', Davenport (1996, 1997, 1998) proposed a usable taxonomy based on the behaviour of microbes, rather than on specific names. Members of Group 1 were

Abbreviation: a_w, water activity.

The EMBL accession number for the sequence of the 26S rDNA D1/D2 region of NCYC 3013^T is AJ310447.

defined as spoilage organisms, proliferating in soft drinks and able to cause spoilage from as few as one cell per container (van Esch, 1987; Davenport, 1996). Group 1 spoilage yeasts were characteristically osmotolerant and resistant to preservatives, such as acetic, sorbic or benzoic acids. Molecular taxonomic methods have shown that most of the Group 1 yeasts are closely related, clustered around the *Zygosaccharomyces sensu stricto* clade (James *et al*., 1994). Members of Group 2 were described as spoilage/hygiene micro-organisms, capable of causing spoilage of soft drinks following mistakes during manufacture. These are opportunistic spoilage organisms, often present in soft-drinks factories in small numbers. Group 3 microbes were hygiene indicators and would not cause spoilage.

In this paper, a novel species of yeast, isolated at a soft-drinks production facility, is reported and its spoilage characteristics are defined. The name *Candida davenportii* sp. nov. is proposed for this novel species.

METHODS

Yeast strains. The yeast strains used in this work are listed in Table 1. These include *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* (Group 1; Davenport, 1996), *Candida parapsilosis* and *Saccharomyces cerevisiae* (Group 2) and *Rhodotorula glutinis* (Group 3). NCYC strains are available from the National Collection of Yeast Cultures, Norwich, UK (http://www.ncyc.co.uk).

Media and growth conditions. Yeasts were maintained at 4 °C on slopes of YEPD agar. YEPD contained (l^{-1} water): glucose, 20 g; bacteriological peptone (Oxoid), 20 g; yeast extract (Oxoid), 10 g, corrected to pH 4·0 using 10 M HCl. Starter cultures comprised 10 ml YEPD broth in 30 ml capped McCartney bottles, cultured at 25 °C for 48 h. Experimental cultures, similarly 10 ml YEPD in 30 ml bottles, were inoculated at 1×10^3 cells ml^{-1} and cultured without shaking at 25 °C for 14 days. Synthetic soft-drink medium contained (l^{-1} water): glucose, 80 g; citric acid, 3·5 g; ammonium sulphate, 0·5 g; potassium orthophosphate, 0·5 g; yeast extract (Oxoid), 0·5 g. Aliquots (28 ml) of synthetic soft drink were filled into McCartney bottles, leaving 2 ml headspace.

Addition of inhibitors. Yeasts were characterized by resistance to preservatives, inhibitors, osmotolerance and low pH. Resistance was determined by the MIC. All tests were carried out in YEPD medium at pH 4·0. Acetic acid, NaCl and HCl (pH minimum) were added before sterilization. High-sugar media were autoclaved in two parts, sugar and YEPD, to avoid sugar charring.

Scanning electron microscopy. Yeasts were cultured in YEPD medium pH 4·0 for 5 days at 25 °C. Cells were harvested by centrifugation for 5 min at 3000 ***g***, washed three times in citrate buffer (pH 4·0, 50 mM) and resuspended in cacodylate buffer (pH 7·2, 72·5 mM). Cells were fixed in 2·5 % glutaraldehyde for 1 h, washed twice in buffer and resuspended in 50 % (v/v) ethanol. Fixed cells were dehydrated in 60, 70, 80 and 90 % ethanol, each for 10 min, followed by 100 % ethanol, three times. Cells were packaged into ethanol-saturated filter paper, placed in acetone and critical-point dried with carbon dioxide. Cells were then mounted on tape, sputter coated with platinum and examined by SEM.

Yeast identification and 26S rDNA sequencing. Yeasts were identified initially using standard API kits in conjunction with the yeast identification program of Barnett (1996). rDNA sequence analysis was subsequently carried out. The variable D1 and D2 domains of 26S rDNA were PCR-amplified directly from individual yeast colonies following the protocol detailed by James *et al*. (1994) using the conserved fungal oligonucleotide primers NL1 and NL4 (O'Donnell, 1993). Amplified 26S rDNA D1/D2 PCR products were purified using a Qiagen QIAquick PCR purification kit and sequenced directly using a *Taq* DyeDeoxy terminator cycle sequencing kit (PE Biosystems) and an Omnigene thermal cycler (Hybaid). 26S rDNA sequences were determined using NL1 and NL4 as sequencing primers. Purified sequence reaction mixtures were electrophoresed with a PE Biosystems model 373A automated DNA sequencer.

To determine the species identity of strain NCYC 3013^T, the 26S rDNA D1/D2 sequence was used to search against sequences held in both the EMBL and GenBank databases. A sequence alignment of the 26S rDNA sequences for NCYC 3013^T and its closest relatives was created 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 (phylogeny inference package; 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 (using *Candida blankii* as outgroup) was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the NEIGHBOR program. The stability of individual branches of the tree was assessed using the bootstrap method (Felsenstein, 1985) with the programs SEQBOOT, DNADIST,

Table 1. Yeast strains used in this study and their sources

Strain	Source
Candida davenportii NCYC 3013^T	Wasp, soft-drinks plant, The Netherlands
Candida parapsilosis strain 69	Spoiled fruit juice (infant formulation), UK
Rhodotorula glutinis strain 92	Soft-drinks factory, Israel
Saccharomyces cerevisiae NCYC 957	X2180-1B
Zygosaccharomyces bailii NCYC 1766	Spoiled blackcurrant and grape juice, UK
Zygosaccharomyces rouxii NCYC 381	Cane sugar

NEIGHBOR and CONSENSE. A total of 488 nt was determined from the 26S rDNA D1/D2 region of NCYC 3013^T.

RESULTS AND DISCUSSION

Isolation and identification of a novel yeast species

A dead wasp, tentatively identified as *Vespula vulgaris*, was found beside a few drops of sugar syrup at the sampling tap of an external sugar-syrup tank at a European soft-drinks production facility. Several other wasps were seen in the area, attracted by the sugar. A yeast isolated from the wasp could not be identified using standard methods (API kit) and was termed isolate 220^T. Microscope examination of isolate 220^T (*C. davenportii* sp. nov. NCYC 3013^T) showed that the cells were small and ovoid, approximately 2–3 μm in length and 1·2–1·5 μm in diameter. Cell division occurred by budding (Fig. 1). Standard biochemical assimilation tests were carried out on isolate 220^T. Results are shown in Table 2. Isolate 220^T fermented glucose only and grew on glucose, sucrose and raffinose.

The 26S rDNA D1/D2 nucleotide sequence of isolate 220^T was used to search the EMBL and GenBank databases to establish the identity of this yeast isolate at the species level. Results from the search revealed that the 26S rDNA D1/D2 sequence was most similar to that of *Candida stellata*, displaying 94·1 % sequence identity. However, such a level of sequence divergence (5·9 %) indicated that isolate 220^T belonged to a separate and hitherto undescribed yeast species; conspecific strains typically differ by less than 1 % in this region (Kurtzman & Blanz, 1998).

To establish the taxonomic position of this novel species, 26S rDNA D1/D2 sequences of NCYC 3013^T, *C. stellata* and a number of related *Candida* species (Kurtzman & Robnett, 1998) were aligned and used to generate a phylogenetic tree using the neighbour-joining method (Saitou & Nei, 1987). Fig. 2 depicts the phylogenetic placement of NCYC 3013^T in relation to *C. stellata* and other *Candida* species. *C. davenportii* NCYC 3013^T, along with *Candida apicola*, *Candida bombi*, *Candida etchellsii*, *Candida floricola*, *Candida lactis-condensi*, *C. stellata*, *Candida batistae*, *Candida powellii* and *Starmerella bombicola* (*Candida bombicola*), formed a statistically significant species group (bootstrap value of 100 %).

Bee/wasp association of *C. davenportii* and related species

C. davenportii NCYC 3013^T was isolated from *V. vulgaris*, the common wasp (Else, 1994). This finding in isolation does not prove that *C. davenportii* is wasp-associated. Isolation of a strain from a particular location may or may not indicate the normal environment of that strain. However, when the origin of species most closely related to *C. davenportii* was examined, a more convincing picture emerged. Of the 18 species listed in Fig. 2, 11 have been isolated from insects, with ten records of isolation from bees. Besides *C. davenportii* NCYC 3013^T, species in this group noted for having been isolated from insects are *C. bombi* (bumblebees; Montrocher, 1967), *C. apicola* (bees; Hajsig, 1958), *C. stellata* (fruit fly; Spencer *et al.*, 1992), *Candida magnoliae* (bees; Deak & Beuchat, 1993), *Candida gropengiesseri* (cockroach cocoon;

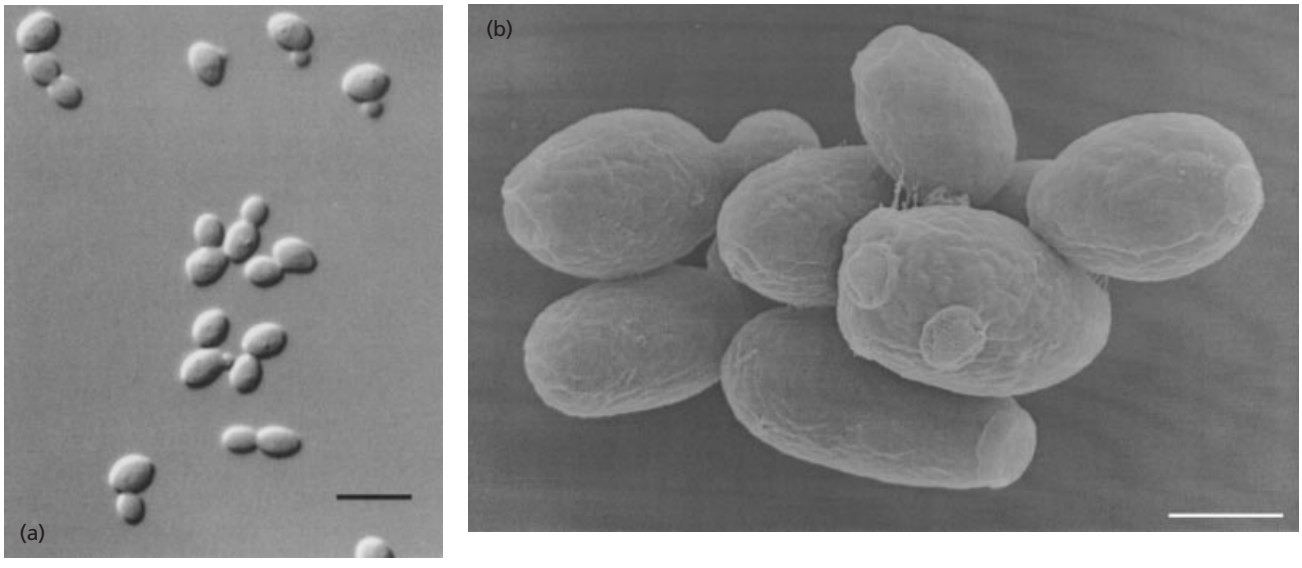

Fig. 1. (a) Photomicrograph of *C. davenportii* sp. nov. NCYC 3013^T grown in yeast nitrogen base medium (25 mM glucose) for 1 day at 25 °C. Bar, 10 μm. (b) Scanning electron micrograph of a small cluster of cells of *C. davenportii* sp. nov. NCYC 3013^T grown for 5 days in YEPD medium pH 4·0 at 25 °C. Bar, 1 μm.

Table 2. Standard physiological and biochemical characteristics of *C. davenportii* sp. nov. NCYC 3013^T

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

NT, Not tested.

Harrison, 1928, quoted by Barnett *et al*., 2000), *Candida apis* (bees; Lavie, 1954, quoted by Barnett *et al*., 2000), *C. batistae* (solitary nesting digger bees; Rosa *et al*., 1999), *C. powellii* and *Candida tilneyi* (bees and nitidulid beetles; Lachance *et al*., 2001a) and *S. bombicola* as *C. bombicola* (bumblebees and alfalfa leaf-cutter bees; Spencer *et al*., 1970; Inglis *et al*., 1993; Rosa & Lachance, 1998). Three novel species related to *C. etchellsii* were also isolated from bees and beetles (Lachance *et al*., 2001b). This evidence suggests strongly that the majority of yeasts in this clade, possibly all species, are primarily associated with Aculeates (bees and wasps). It may be speculated that insects may form the normal environment for these yeasts. Limited data, from *C. bombicola* (Inglis *et al*., 1993), suggest that these yeasts are not carried within the insect digestive system, as has been reported for fruit flies (Lachance *et al*., 1995), but associated with nectar and pollen.

Physiology of *C. davenportii* sp. nov. NCYC 3013^T

C. davenportii NCYC 3013^T grew well in a fruit-juice-containing soft drink (pH 3·3), a synthetic soft drink (pH 3·3) and a cola-type beverage (pH 2·65) and was therefore shown to have the potential to cause spoilage of soft drinks. Spoilage yeasts are characteristically resistant to preservatives and osmotolerant. The preservative resistance of NCYC 3013^T was therefore determined and compared with other, more notorious

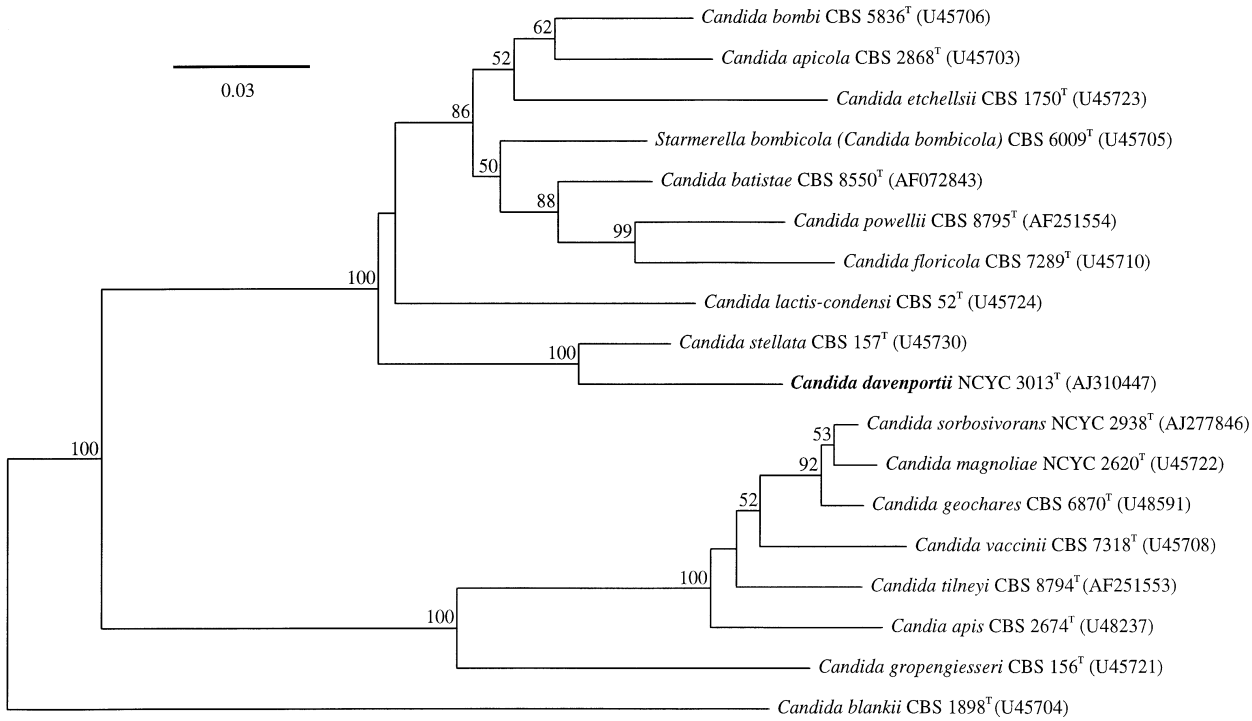

Fig. 2. Dendrogram showing the phylogenetic relationship of *C. davenportii* sp. nov. NCYC 3013^T (isolate 220^T) to other related *Candida* species (Kurtzman & Robnett, 1998; Lachance *et al.*, 2001a) based on 26S rDNA D1/D2 sequences. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values, expressed as percentages of 100 replications, are given at branch points (only values $>$ 50% are shown). Bar, 3 estimated base substitutions per 100 nucleotides. EMBL/GenBank accession numbers are shown in parentheses.

Table 3. Preservative resistance in *C. davenportii* sp. nov. compared with that of other spoilage yeasts

Values are means of at least two determinations of MIC, measured in YEPD, at pH 4·0, following incubation at 25 °C for 14 days.

Preservative	*C. davenportii* NCYC 3013^T	*C. parapsilosis* strain 69	*R. glutinis* strain 92	*S. cerevisiae* NCYC 957	*Z. bailii* NCYC 1766	*Z. rouxii* NCYC 381
Sorbic acid (mM)	1·8	2·4	0·3	3·3	7·6	1·8
Benzoic acid (mM)	2·4	3·1	1·2	3·3	8·7	3·4
Acetic acid (mM)	190	85	45	125	470	135
Ethanol (M)	1·55	1·20	0·60	2·00	1·80	1·60

spoilage yeasts. Results are shown in Table 3. *Z. bailii* showed phenomenal resistance to all acidic preservatives. *C. davenportii* NCYC 3013^T was moderately resistant to sorbic and benzoic acids, but less resistant than *C. parapsilosis*, *S. cerevisiae* or *Z. rouxii*. However, *C. davenportii* NCYC 3013^T was unexpectedly resistant to acetic acid, with an MIC value of 190 mM. While not approaching the resistance of *Z. bailii* to acetic acid (Table 3), NCYC 3013^T was more resistant to acetic acid than almost all other spoilage yeasts.

C. davenportii NCYC 3013^T was osmotolerant and capable of growth in up to 3·3 M glucose (59·4%, w/v). This is comparable with other spoilage yeasts, with the exception of *Z. rouxii* and *Z. bailii*. *C. davenportii* NCYC 3013^T was, however, substantially less salt tolerant than other spoilage yeasts, being inhibited by 1·7 M NaCl (10%, w/v). Almost all species related to NCYC 3013^T are also sugar tolerant, most species being able to grow in 60% (w/v) glucose (Barnett *et al.*, 2000). Recognized osmotolerant yeasts in this clade include *C. apicola*, *C. etchellsii*, *C. lactis-condensi* and *C. magnoliae*, with a minimum water activity (a_w) for growth of 0·70 in sucrose/glycerol syrups (Tilbury, 1980). It is possible that osmotolerance may aid survival of these yeasts in low-a_w habitats associated with bee/wasp nectar or honey.

Unusual tolerance to low pH was shown by *C.*

davenportii NCYC 3013^T. *Z. bailii* NCYC 1766 and *Z. rouxii* NCYC 381 failed to grow in media at pH 2·0, whereas *C. davenportii* NCYC 3013^T grew well in YEPD media down to an initial pH of 1·4. While growth of most yeasts is impaired at pH 3·0, *C. davenportii* NCYC 3013^T grew almost as well at pH 2·0 as at pH 3·0, suggesting that growth at pH 1·8–2·0 was, unusually, not stressful to this species. Other related species showed only average resistance to low pH: *C. stellata* NCYC 486, pH minimum 2·1; *C. etchellsii* NCYC 2432, pH minimum 1·9.

Spoilage significance of *C. davenportii* NCYC 3013^T and related species

Many of the yeasts related to *C. davenportii* NCYC 3013^T have been implicated in spoilage of foods, particularly sugary, low-a_w foods. Spoilage of high-sugar commodities has been reported by *C. apicola* (blackcurrant drink, sugar syrups; Hajsig, 1958; Scarr & Rose, 1966), *C. bombicola* (concentrated grape juice, high-sugar vegetables; Spencer *et al.*, 1970; Ok & Hashinaga, 1997), C. *etchellsii* (concentrated citrus juice; Recca & Mrak, 1952), *C. lactis-condensi* (condensed milk, sugar syrups; Hammer, 1919; Scarr & Rose, 1966), *C. stellata* (soft drinks, fruit juices and concentrates, tomato sauce; Pitt & Richardson, 1973; Sand & van Grinsven, 1976a, b; Spencer *et al.*, 1992; Deak & Beuchat, 1993) and *C. magnoliae* (concentrated fruit juice; Deak & Beuchat, 1993). Spoilage of high-salt commodities by *C. etchellsii*, *C. apicola* and *C. lactis-condensi* has been reported (Tilbury, 1976, 1980; Barnett *et al.*, 2000). Spoilage by these yeasts is relatively uncommon and, in the context of spoilage significance, they are probably Group 2, opportunist spoilage microbes (Davenport, 1996). Pitt & Hocking (1997) do not list these yeasts as responsible for spoilage of foods processed and packaged according to normal standards of good manufacturing practice.

The high-sugar commodities spoiled by these yeasts include condensed milk, concentrated fruit juices and sugary syrups, all likely to attract bees and wasps. Workers of the common wasp, *V. vulgaris*, are commonly a great nuisance in late summer in various food factories, confectionery shops, greengrocers, fish shops, at picnics and wherever sugary foodstuffs are exposed (Else, 1994). It is therefore a distinct possibility that spoilage of sugary foods by *C. davenportii* and related species occurs when bees and wasps carry the yeast infection to the food, bees and wasps thus being the direct source of infection of these spoilage yeasts.

The novel yeast species is named *C. davenportii* sp. nov. in honour of Professor Bob Davenport, whose work with spoilage yeasts in the soft-drinks production environment is legendary. It was with deep sadness that the authors learned of the death of Professor Davenport in 2001, shortly after he had been informed of the discovery and naming of this novel yeast species.

Latin diagnosis of *Candida davenportii* Stratford, Bond, James, Roberts & Steels sp. nov.

Cultura in agaro morphologico (Difco) *post* 48 *horas ad* 24 °C: *cellulae ovoideae* (2·0–3·0 × 1·0–1·5 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*. *In agaro farinae Zea mays post dies* 14 *pseudomycelium non formatur*. *Glucosum fermentantur at non sucrosum maltosum*, *galactosum*, *lactosum*, *cellobiosum*, *trehalosum*, *melibiosum*, *melezitosum*, *raffinosum*, *methyl* α-D-*glucosidum*, *inulinum nec amylum*. *Glucosum*, *sucrosum*, *raffinosum et lysinum assimilantur at non galactosum*, L-*sorbosum*, *maltosum*, *cellobiosum*, *trehalosum*, *lactosum*, *melibiosum*, *inulinum*, *amylum*, D-*xylosum*, L-*arabinosum*, D-*arabinosum*, D-*ribosum*, L-*rhamnosum*, *galactitolum*, *methyl* α-D-*glucosidum*, *melezitosum*, *erythritolum*, *ribitolum*, D-*mannitolum*, D-*glucitolum*, *salicinum*, *acidum succinicum*, *acidum citricum*, *glucono*-D-*lactonum*, *glycerinum*, *xylitolum*, *nitrus kalicus*, *ethylaminum*, *acidum lacticum*, *inositolum*, D-*glucosaminum*, *cadaverinum*, *methanolum nec alcohol aethylicum*. *Crescit in medio cum* 50 % *glucoso*. *Non crescit in medio* 1 % *acido acetico addito et in medio* 0·01 % *cycloheximido addito*. *Typus depositus in zymotica collectionis* National Collection of Yeast Cultures, Norwich, UK (NCYC 3013^T) *et* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 9069^T).

Description of *Candida davenportii* Stratford, Bond, James, Roberts & Steels sp. nov.

Candida davenportii (L. gen. sing. masc. n. *davenportii* of Davenport, referring to Robert R. Davenport, in recognition of his life-long work on spoilage yeasts in the soft-drinks environment).

On morphology agar, after 48 h at 24 °C, cells are spherical to ovoid (2·0–3·0 × 1·0–1·5 μm) and occur singly, in pairs or in groups (Fig. 1). Budding is multilateral. No ascosporulation is observed after incubation for 3 weeks at 24 °C, on corn-meal agar, potato-dextrose agar or Gorodkowa agar. Pseudohyphae are not formed. A summary of the physiological and other growth characteristics of *Candida davenportii* sp. nov. NCYC 3013^T is given in Table 2. Cultures of the type strain, strain 220^T, have been deposited with the National Collection of Yeast Cultures, Norwich, UK (NCYC 3013^T), and the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 9069^T).

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr James Barnett, School of Biological Sciences, University of East Anglia, and Linda Barnett in the preparation of photomicrographs and Mark Kirkland at Unilever Research, Colworth House, for the scanning electron micrograph. The assistance of Barbara C.

Last is gratefully acknowledged in the taxonomy and identification of wasps.

REFERENCES

Barnett, J. A. (1996). Yeast Identification PC program: version 4. ISBN 0951314882.

Barnett, J. A., Payne, R. W. & Yarrow, D. (2000). *Yeasts: Characteristics and Identification*, 3rd edn. Cambridge: Cambridge University Press.

Byrne, M. (1994). The beverage boom continues. *Food Eng Int* **19**, 52, 54–57.

Davenport, R. R. (1996). Forensic microbiology for soft drinks business. *Soft Drinks Int* April 1996, 34–35.

Davenport, R. R. (1997). Forensic microbiology II. Case book investigations. *Soft Drinks Int* April 1997, 26–30.

Davenport, R. R. (1998). Microbiology of soft drinks. In *The Chemistry and Technology of Soft Drinks & Fruit Juices*, pp. 197–216. Edited by P. R. Ashurst. Sheffield: Sheffield Academic Press.

Deak, T. & Beuchat, L. R. (1993). Yeasts associated with fruit juice concentrates. *J Food Prot* **56**, 777–782.

Else, G. (1994). Social wasps. *Brit Wildl* **5**, 304–311.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5. Department of Genetics, University of Washington, Seattle, USA.

Feng, D. F. & Doolittle, R. F. (1987). Progressive sequence alignment as a prerequisite to correct phylogenetic trees. *J Mol Evol* **25**, 351–360.

Genetics Computer Group (1991). *Program Manual for the GCG Package*, *Version 7*. Madison, WI: Genetics Computer Group.

Goverd, K. A., Beech, F. W., Hobbs, R. P. & Shannon, R. (1979). The occurrence and survival of coliforms and salmonellas in apple juice and cider. *J Appl Bacteriol* **46**, 521–530.

Hajsig, M. (1958). *Torulopsis apicola* nov. spec., new isolates from bees. *Antonie Leeuwenhoek* **24**, 18–22.

Hammer, B. W. (1919). Studies on the formation of gas in sweetened condensed milk. *Iowa Agric Exp Stn Res Bull* **54**, 211–220.

Harrison, F. C. (1928). A systematic study of some torulae. *Trans R Soc Can* **21**, 341–380.

Inglis, G. D., Sigler, L. & Goettel, M. S. (1993). Aerobic microorganisms associated with alfalfa leafcutter bees (*Megachile rotundata*). *Microb Ecol* **26**, 125–143.

James, S. A., Collins, M. D. & Roberts, I. N. (1994). Genetic interrelationship among species of the genus *Zygosaccharomyces* as revealed by small-subunit rRNA gene sequences. *Yeast* **10**, 871–881.

Kurtzman, C. P. & Blanz, P. A. (1998). Ribosomal RNA/DNA sequence comparisons for assessing phylogenetic relationships. In *The Yeasts*, *a Taxonomic Study*, 4th edn, pp. 69–74. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Leeuwenhoek* **73**, 331–371.

Lachance, M. A., Gilbert, D. G. & Starmer, W. T. (1995). Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: a comparison with western communities. *J Ind Microbiol* **14**, 484–494.

Lachance, M.-A., Bowles, J. M., Chavarría Díaz, M. M. & Janzen, D. H. (2001a). *Candida cleridarum*, *Candida tilneyi* and *Candida powellii*, three new yeast species isolated from insects associated with flowers. *Int J Syst Evol Microbiol* **51**, 1201–1207.

Lachance, M. A., Starmer, W. T., Rosa, C. A., Bowles, J. M., Barker, J. S. F. & Janzen, D. H. (2001b). Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Res* **1**, 1–13.

Lavie, P. (1954). Essais de lutte biologique contre l'Acarapis woodi, agent de l'acariose d'abeille. *C R Acad Sci Hebd Seances Acad Sci* **238**, 947–948.

Montrocher, R. (1967). Quelques nouvelles especes et varietes du genre *Candida* (levures asporogenes). *Rev Mycol* **32**, 69–92.

O'Donnell, K. (1993). *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic*, *Meiotic and Pleomorphic Speciation in Fungal Systematics*, pp. 225–233. Edited by D. R. Reynolds & J. W. Taylor. Wallingford: CAB International.

Ok, T. & Hashinaga, F. (1997). Identification of sugar-tolerant yeasts from high-sugar fermented vegetable extracts. *J Gen Appl Microbiol* **43**, 39–47.

Pitt, J. I. & Hocking, A. D. (1997). *Fungi and Food Spoilage*, 2nd edn. London: Blackie Academic & Professional.

Pitt, J. I. & Richardson, K. C. (1973). Spoilage by preservative-resistant yeasts. *CSIRO Food Res Q* **33**, 80–85.

Recca, J. & Mrak, E. M. (1952). Yeasts occurring in citrus products. *Food Technol* **6**, 450–454.

Rosa, C. A. & Lachance, M.-A. (1998). The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. *Int J Syst Bacteriol* **48**, 1413–1417.

Rosa, C. A., Viana, E. M., Martins, R. P., Antonini, Y. & Lachance, M. A. (1999). *Candida batistae*, a new yeast species associated with solitary digger nesting bees in Brazil. *Mycologia* **91**, 428–433.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sand, F. E. M. J. (1970). Zur hefe-flora von erfrischungsgetranken. *Brauwelt* **110**, 225–236.

Sand, F. E. M. J. (1971). Zur hygiene von abfullkolonnen der erfrischungsgetranke-industrie. *Brauwelt* **111**, 1788–1800.

Sand, F. E. M. J. (1973). Recent investigations on the microbiology of fruit juice concentrates. In *Technology of Fruit Juice Concentrates – Chemical Composition of Fruit Juices*, pp. 185–216. Edited by Scientific-Technical Commission XIII. Vienna: International Federation of Fruit Juice Producers.

Sand, F. E. M. J. & van Grinsven, A. M. (1976a). Investigation of yeast strains isolated from Scandinavian soft drinks. *Brauwissenschaft* **29**, 353–355.

Sand, F. E. M. J. & van Grinsven, A. M. (1976b). Comparison between the yeast flora of Middle Eastern and Western European soft drinks. *Antonie Leeuwenhoek* **42**, 523–532.

Scarr, M. P. & Rose, D. (1966). Study of osmophilic yeasts producing invertase. *J Gen Microbiol* **45**, 9–16.

Spencer, J. F. T., Gorin, P. A. J. & Tulloch, A. P. (1970). *Torulopsis bombicola* sp. n. *Antonie Leeuwenhoek* **36**, 129–133.

Spencer, D. M., Spencer, J. F. T., de Figueroa, L. & Heluane, H. (1992). Yeasts associated with rotting citrus fruits in Tucuman, Argentina. *Mycol Res* **96**, 891–892.

Stratford, M., Hofman, P. D. & Cole, M. B. (2000). Fruit juices, fruit drinks and soft drinks. In *The Microbiological Safety and Quality of Food*, pp. 836–869. Edited by B. M. Lund, A. C. Baird-Parker & G. W. Gould. Gaithersburg, MD: Aspen Publishers.

Tilbury, R. H. (1976). The microbial stability of intermediate moisture foods with respect to yeasts. In *Intermediate Moisture Foods*, pp. 138–165. Edited by R. Davies, G. G. Birch & K. L. Parker. London: Applied Science.

Tilbury, R. H. (1980). Xerotolerant (osmophilic) yeasts. In *Biology and Activity of Yeasts*, Society for Applied Bacteriology Symposium Series, pp. 153–179. Edited by F. A. Skinner, S. M. Passmore & R. R. Davenport. London: Academic Press.

van Esch, F. (1987). Yeasts in soft drinks and fruit juice concentrates. *De Ware*(*n*) *Chemicus* **17**, 20–31 [English reprint in *Brygemeteren* **4** 9–20].

Zhao, T., Doyle, M. P. & Besser, R. E. (1993). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. *Appl Environ Microbiol* **59**, 2526–2530.