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Dekkera anomala sp. nov., the teleomorph of *Brettanomyces anomalus*, recovered from spoiled soft drinks

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A new ascogenous yeast species, *Dekkera anomala*, is described. The strains studied were isolated from spoiled soft drinks. This species differs morphologically and physiologically from the species presently accepted in the genus *Dekkera* by the formation of blastese and the ability to ferment lactose.

INTRODUCTION

Some yeast isolates from spoiled carbonated, fruit-based soft drinks were found a) to produce hat-shaped ascospores which are liberated soon after maturation and tend to agglutinate, b) to form ogival cells, c) to produce aerobically marked amounts of acetic acid from glucose and d) to require an extraneous vitamin source. On the basis of these characteristics, the strains might be placed in the genus *Dekkera* van der Walt. Morphologically as well as physiologically these strains cannot be identified with any of the two species accepted thus far.

MATERIALS AND METHODS

Strains examined were CBS 8138 and CBS 8139, isolated from spoiled carbonated, fruit-based soft drinks by the Microbiological Department of Naarden International Holland B.V.

The description of the new species is based on the standard methods applied in yeast taxonomy (Van der Walt, 1970a). For growth tests, performed in tripli-

cate, cultures were incubated on a shaker for 21 days at 25° C. Utilization of nitrogen sources was examined in auxanograms for 7 days. Acetic acid production was detected by the smell of the cultures on malt agar and confirmed in malt extract with a test kit (Boehringer). The Diazonium Blue B (DBB) test was performed according to the method described by Van der Walt and Hopsu-Havu (1976). Deoxyribonucleic acid was purified, the DNA base composition was determined and the ascigerous material for ultra-thin sections was prepared according to the methods previously described (Smith et al., 1981).

Description

Dekkera anomala M. Th. Smith et van Grinsven sp. nov.
Stat. anamorphosis: *Brettanomyces anomalus* Custers 1940

In extracto malti post dies 5 25° C, cellulae rotundae, ovoideae, saepe ogivales, cylindricae elongataeque, (2.0–4.5) × (3.5–22.0) µm singulae, binae vel catenatae. Cellulae longae pseudomycelii et interdum cellulae valde elongatae ramosae (blastesis) formantur. Sedimentum adest. In agaro Wickerhamii post dies 5 25° C, cellulae forma aequales atque in extracto malti, (2.0–5.0) × (3.5–26.0) µm. Cultura alba vel albiflava, nitida, glabra vel undulata. Margo glaber vel lobatus. Pseudohyphae e cellulis elongatis consistunt cum blastosporis ellipsoideis cylindricisque. Cellulae valde elongatae ramosae nonseptatae adsunt. Asci e cellulis diploideis transformatis oriuntur. Ascospores pileiformes ad 4 in quoque asco formantur; ascosporae maturae ex ascis celeriter liberantur et inter se agglutinantur. Fermentatio, crescentia in variis substantiis carbonaceis et alii characteres monstrantur in Tabula 1.
Typus: CBS 8139 isolatus e suco citri, vivus et exsiccatus in collectione Centraalbureau voor Schimmelcultures, Delphis Batavorum et Baarn.

Growth in malt extract: After 5 days at 25° C the cells are spheroidal, usually ellipsoidal, frequently ogival (Fig. 1), cylindrical to elongate, (2.0–4.5) × (3.5–22.0) µm, singly, in pairs or short chains. Pseudomycelium is usually formed. Filamentous, frequently branching, non-septate cells which resemble non-septate hyphae are also produced. A floccose to mucoid sediment is formed. Pellicle formation is absent. A characteristic aroma usually develops.

Growth on Wickerham's morphology agar: After 5 days at 25° C the cells are very similar to those in malt extract, (2.0–5.0) × (3.5–26.0) µm. After 2 weeks at room temperature the streak is white to cream-coloured, glistening, smooth to undulate. The margin is entire to slightly lobate.

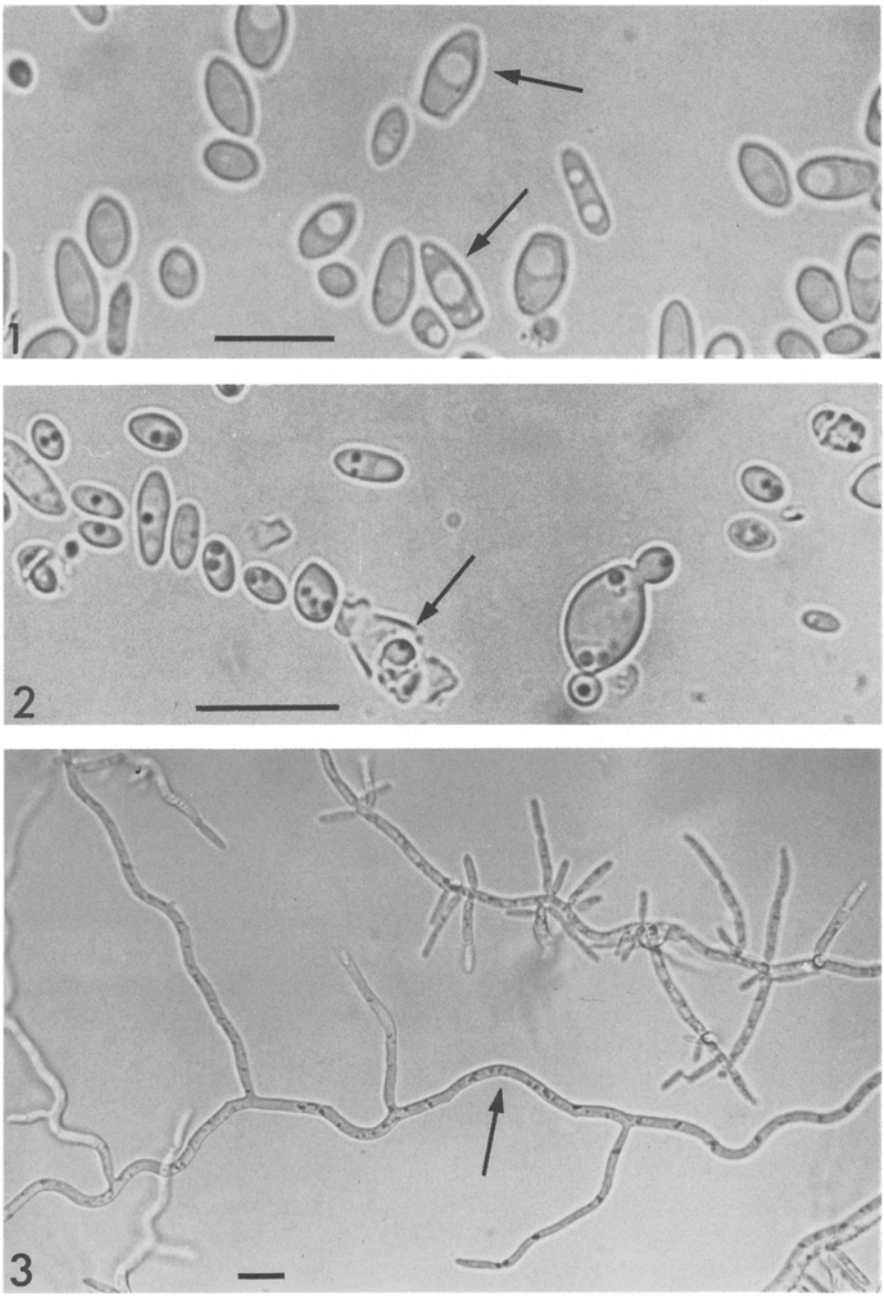
Growth on malt agar + 2% calcium carbonate: After 6 weeks at room temperature the streak is restricted, slightly raised, white to cream-coloured, somewhat

Fig. 1. *Dekkera anomala* CBS 8138. Ogival cells (arrow) in malt extract after 5 days.

Fig. 2. *Dekkera anomala* CBS 8138. Hat-shaped ascospores in a disintegrating ascus (arrow). ▶

Fig. 3. *Dekkera anomala* CBS 8139. Pseudohyphae and non-septate hyphae (arrow) on potato agar.

Bars represent 10 µm.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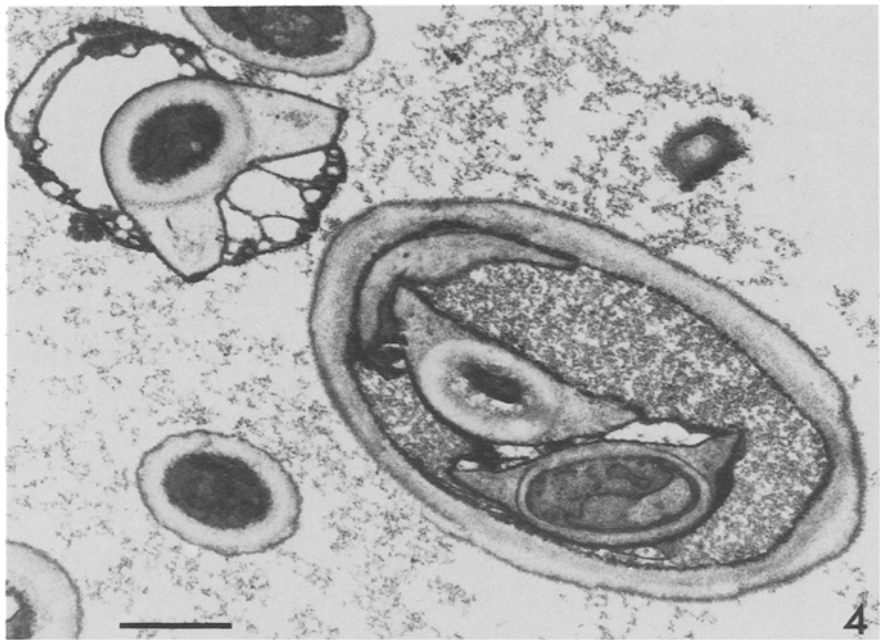

Fig. 4. *Dekkera anomala* CBS 8139. Electron micrograph of an ultrathin section of an ascus with hat-shaped ascospores. Potassium-permanganate fixation.
Bar represents 1 μm.

shiny to dull, smooth or rugose. The margin may be entire, lobate or fringed with mycelium. Acid is produced.

Growth in slide cultures on potato-, rice- and morphology-agar: After 5 days at 25°C, pseudomycelium is produced. It consists of filamentous cells along which ellipsoidal to cylindrical blastoconidia are formed, singly or in short chains, not arranged in characteristic clusters or verticils. Slender, non-septate, filamentous cells (diameter 1.5–3.5 μm) which often branch and resemble a ramified mycelium (blastese) are formed (Fig. 3).

Formation of asci and ascospores: Vegetative cells are directly transformed into unconjugated evanescent asci with one to four ascospores (Fig. 2). Mature ascospores which tend to agglutinate when liberated, are galeate (Fig. 4). Ascospore formation is observed on 3% Difco malt agar after 3 weeks; it may be sparse.

Physiological characteristics: Fermentation and growth on various carbon compounds, growth on nitrogen sources and some additional properties are summarized in Table 1.

Salient characteristics: a) Short life of the cultures unless frequently trans-

Table 1. Physiological characteristics of *Dekkera anomala* sp. nov.

Fermentation	D-glucose	+	α,α-trehalose	+	raffinose	−
	D-galactose	+	α-methyl-D-glucoside	+	melezitose	+
	D-xylose	−	cellobiose	+	soluble starch	−
	sucrose	+	melibiose	−		
	maltose	+	lactose	+		
Growth	D-glucose	+	cellobiose	+	D-mannitol	−
	D-galactose	+	salicin	+	galactitol	−
	L-sorbose	−	arbutin	+	inositol	−
	D-glucosamine	+	melibiose	−	D-glucono 1,5-lactone	+
	D-ribose	−	lactose	+	2-keto-D-gluconate	−
	D-xylose	−	raffinose	+	D-gluconate	−
	L-arabinose	−	melezitose	+	DL-lactate	−
	D-arabinose	−	inulin	−	succinate	−
	L-rhamnose	−	soluble starch	−	citrate	−
	sucrose	+	glycerol	+	methanol	−
	maltose	+	erythritol	−	ethanol	+
	α,α-trehalose	+	ribitol	−		
	α-methyl-D-glucoside	+	D-glucitol	−		
	nitrate	+				
	nitrite	+				
	ethylamine	+				
	50% glucose	−				
	37°C	+				
	0.1% cycloheximide	+				
	vitamin-free medium	−				
Additional characteristics	starch formation	−				
	acid production on Custers' medium	+				
	G + C%	38.4 (CBS 8138) 38.5 (CBS 8139)				
	Diazonium Blue B test	−				

ferred or unless calcium carbonate is added to the medium, and b) production of acetic acid from glucose under aerobic conditions.

Type culture: CBS 8139 isolated from spoiled soft drinks, deposited in the collections of the Centraalbureau voor Schimmelcultures at Delft and Baarn, living and dried, respectively.

Discussion

In the genus *Dekkera* two species are recognized by Van der Walt (1970*b*): *Dekkera bruxellensis* van der Walt and *Dekkera intermedia* van der Walt. The new species *Dekkera anomala* can be distinguished from these species: (1) mor-

phologically by the characteristic production of blastese, as defined by Custers (1940) and Van der Walt (1970*c*) as slender, non-septate, filamentous cells which often branch and resemble a ramified, one-celled mycelium, and (2) physiologically by its ability to ferment lactose. Both characteristics are considered to be of value in the classification of *Brettanomyces,* the anamorph of *Dekkera.* The new species further differs from *D. bruxellensis* by utilizing galactose and the β-glucosides cellobiose, salicin and arbutin. Because the new taxon is identical with *Brettanomyces anomalus* morphologically and physiologically, it may be considered the teleomorph of this species. Therefore, the epithet anomala was chosen.

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