***Sterigmatomyces acheniorum* species nova, a Yeast from Strawberries**

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SUMMARY

A new species of yeast, *Sterigmatomyces acheniorum*, is described. The strains, isolated from fresh strawberries, differed from other species of *Sterigmatomyces* in the test substrates they utilized. The strains grew slowly on certain organic substrates on three of which they formed mainly small colonies with a minority of large ones. When subcultured on the same substrates, large colonies gave rise to further large colonies, and small colonies produced mainly small colonies with a minority of large ones.

INTRODUCTION

During a recent survey of yeasts associated with fresh strawberries (Buhagiar & Barnett, 1971), a strain of a kind hitherto undescribed was isolated in 1969; a further five similar strains were isolated in 1970 and another in 1971. Four were isolated at 15 °C and the others at 20 °C and 25 °C, all from surface colonies on Difco Bacto YM agar at pH 3.4 or 4.8. The present paper describes the new species.

METHODS

The methods used have been described by Barnett & Buhagiar (1971) and Buhagiar & Barnett (1971).

RESULTS

Macroscopical appearance

Streak cultures on Difco Bacto YM agar at 25 °C were creamy white and semiglossy after 2 or 3 days; cream to buff coloured with well defined margins, flat, matt or semiglossy after one week; cream or buff, matt, granular, with pale striated margins and well defined lobate edges after one month. Cultures may be pink on some samples of malt extract agar (D. Yarrow, 1972, personal communication).

Microscopical appearance

In exponential growth, the cells were ellipsoidal, elongate or pear-shaped and measured $4.4 \times 2.7 \mu\text{m}$ (mean of 382 cells of five strains; mode $4.2 \times 2.6 \mu\text{m}$; maxima $7.9 \times 4.0 \mu\text{m}$; minima $2.6 \times 1.6 \mu\text{m}$; see Table 1). Length and breadth were not highly correlated. Buds were usually formed on sterigmata, of which each cell put out one, two or three simultaneously. After reaching about $1 \mu\text{m}$ in length, each sterigma formed a distal, spherical swelling which was a new bud (cf. Fell, 1970; see Fig. 1). Each bud enlarged and lengthened,

Table 1. *Measurements of cells of five strains of Sterigmatomyces acheniorum harvested in exponential growth*
 The yeasts were cultured aerobically in defined liquid medium with D-glucose as sole carbon source, examined microscopically and photographed.
 Photographic prints of the cells ($\times 1900$) were measured to 0.5 mm ($\equiv 0.26 \mu\text{m}$).

Strain	No. of cells measured	Length (μm)			Breadth (μm)			<i>r</i>		
		Mean and standard deviation	Maximum	Minimum	Mode	Mean and standard deviation	Maximum		Minimum	Mode
CBS 6385	80	3.8 $\pm$ 0.6	6.3	2.6	3.7	2.5 $\pm$ 0.3	3.4	1.8	2.6	+0.40
CBS 6386	49	4.8 $\pm$ 0.9	7.9	3.2	4.5	2.6 $\pm$ 0.5	3.7	1.6	2.6	+0.60
FRI F187	57	4.3 $\pm$ 0.7	5.3	3.2	4.3	2.8 $\pm$ 0.5	3.7	1.8	2.9	+0.77
CBS 6384	100	5.1 $\pm$ 1.1	7.9	2.6	4.7	2.6 $\pm$ 0.3	4.0	1.6	2.6	+0.66
FRI A 24	96	4.0 $\pm$ 0.5	5.3	3.2	4.0	2.8 $\pm$ 0.4	3.7	1.8	2.9	+0.61
All five strains	382	4.4 $\pm$ 0.9	7.9	2.6	4.2	2.7 $\pm$ 0.4	4.0	1.6	2.6	—

r = Correlation coefficient of sample; in every case, $Pr(\rho = 0) < 0.001$, ρ = correlation coefficient of population.

becoming about the same size as the mother-cell from which it then separated. When daughter- and mother-cells separated, the linking sterigmata appeared to break either at the mother-cell or at the daughter-cell or midway between. Occasionally sterigmata did not break and chains of up to four cells were formed, each cell separated from the next by a short sterigma. Most sterigmata were apical or nearly apical; the older ones were thicker, and some were curved, appearing flexible; the buds were seen to move relative to the mother-cell though still fully attached. Each strain produced a number of cells with lateral sterigmata. Half-way along some sterigmata, a swelling occurred from which one or two buds formed. Cells growing in defined media were similar to those in Difco Bacto malt extract (Fig. 2).

There was poor growth under the coverslips in slide cultures; a little primitive pseudomycelium occurred outside the coverslips. No ascospores or ballistospores were formed.

Physiological tests

Semi-anaerobic fermentation. The yeasts did not produce gas in Durham tubes from yeast water with 50 mM-D-glucose.

Aerobic growth tests gave the results shown in Table 2. Growth on ten substrates was delayed or slow when compared to growth on glucose. Fig. 3 shows the growth of one strain on nine of these substrates, namely methyl α -D-glucoside, cellobiose, melibiose, lactose, L-sorbose, D-arabinose, ribitol, D-glucose or inulin.

Other tests. Starch production tests gave negative results; urea was hydrolysed. All strains grew best at about 20 °C; at 30 °C five strains grew poorly and one (A24) not at all. None grew at 37 °C. No evidence for acid production was seen after one week on CaCO₃ plates. No strains grew on media with 50 % (w/v) D-glucose, nor > 11 % (w/v) NaCl.

Fig. 1. Drawing of *Sterigmatomyces acheniorum* during exponential growth in shaken culture of defined liquid medium with D-glucose as sole source of carbon. The drawing was made from photomicrographs of several strains, to illustrate the various forms.

Fig. 2. Photomicrograph of *Sterigmatomyces acheniorum* (CBS 6384) growing aerobically in unshaken Difco Bacto malt extract for 3 days at 25 °C. Leitz phase contrast (Heine).

Slow growths

† Since various interpretations can be given to delayed responses in growth tests (Knight, 1938; Barnett, 1966, 1968*a*), the slow growth of three strains of *Sterigmatomyces acheniorum* on methyl α -D-glucoside, on melibiose and on lactose was examined further.

It was necessary to ensure that the late growths were not a consequence of changes in the media resulting from long incubation. Three lots of uninoculated complete medium, each including methyl α -D-glucoside, melibiose or lactose (all filter-sterilized as usual), were therefore incubated at 25 °C for three months. Growth in such pre-incubated medium was not markedly different from that in fresh medium, and hence non-enzymatic hydrolysis of the glycosides was probably not significant. The growth rate on any substrate was not affected appreciably when the inoculum had been grown on that substrate rather than on glucose. Hence we investigated the possibility that the slow growths could be explained in terms of selection. After plating serial dilutions of each strain on agar medium containing one or

Fig. 3. Growth of *Sterigmatomyces acheniorum* (CBS 6386) on nine test compounds. Test tubes contained initially 5 ml of the chemically defined liquid basal medium (Barnett & Ingram, 1955), 250 μ mole of substrate (as indicated), inoculated with about 5 μ g dry wt of yeast from a slope of Difco Bacto YM agar incubated at 15 °C for 3 days. The tubes were incubated at 20 °C and were rocked through an angle of about 40° to 15° from the horizontal. Each point on the graph corresponds to a single nephelometric observation. ●, D-glucose; ◻, methyl α -D-glucoside; ▲, melibiose; ◊, D-arabinose; ○, inulin; ■, cellobiose; △, lactose; ◆, ribitol; ▽, L-sorbose.

Table 2. Results of aerobic growth tests on *Sterigmatomyces acheniorum*

Substrate	Response	Substrate	Response	Substrate	Response
Raffinose	+	D-Glucose	+	Glycerol	S
Melezitose	+	D-Galactose	+	Erythritol	+
Sucrose	+	L-Sorbose	S	Ribitol	S
Maltose	+	L-Rhamnose	—	D-Glucitol	+
Trehalose	+			D-Mannitol	+
Methyl α -D-glucoside	S	D-Ribose	+	Galactitol	—
		D-Xylose	+		
Cellobiose	S	D-Arabinose	S	myo-Inositol	—
Salicin	—	L-Arabinose	+		
				Starch	—
Melibiose	S	Succinate	+	Inulin	S
		Citrate	+		
Lactose	S	Lactate	S	Nitrate	+
D-glucose (medium without vitamins)	—			YM agar at 30 °C	very little growth
YM agar + 50 % (w/v) D-glucose	—			YM agar at 37 °C	—

+, Growth reached about 0.4 mg dry wt/ml within 7 days; —, no growth; S, growth did not reach 0.4 mg dry wt/ml until after 7 days, with an initial delay of at least 3 days (cf. Barnett & Buhagiar, 1971; Buhagiar & Barnett, 1971).

other of the glycosides or D-glucose (Barnett, 1968*b*), there were no appreciable differences between substrates in numbers of colonies for any one strain. However, although the colonies on medium containing 50 mM-D-glucose as sole carbon source appeared uniform, those on 50 mM methyl α -D-glucoside, melibiose or lactose were mixed, mainly small, with not more than about 10 % large. After 9 days at 20 °C with methyl α -D-glucoside the 'large' colonies were about 3 mm diam. and the 'small' colonies 1 mm diam. (Fig. 4, 5). There were no obvious differences in the size of cells from the two kinds of colony.

Fig. 4. Photograph showing large and small colonies of *Sterigmatomyces acheniorum* (CBS 6386) on defined medium with 50 mM-methyl α -D-glucoside as sole source of carbon after 9 days of incubation at 20 °C. The inoculum was grown on Difco Bacto YM agar.

Colonies of both sizes were picked off, subcultured several times on YM agar and then replated successively on each glycoside. Considering any pair of glycosides, A and B, large colonies from A gave rise to large colonies on A, and mainly small colonies on B. Small colonies from A gave mainly small colonies on either A or B. For example, with strain CBS 6386 on methyl α -D-glucoside, colonies derived from an originally small colony included a minority that were large. Those derived from a large colony were all large. At the second plating, colonies generally tended to be smaller than those from which they originated. Cultures derived from a small colony on a given substrate and grown aerobically on that substrate in agitated liquid medium had a longer lag than did those cultures derived from large colonies (Fig. 6). There was no marked difference in the maximum growth rates.

When the concentration of glycoside was varied between 25 mM and 300 mM, the size distributions of the colonies, plated directly from the original stock cultures, showed differences comparable to those described above, though the colonies were larger when grown on higher concentrations of substrate.

Fig. 5

Fig. 6

Fig. 5. Histogram showing distribution of size of 100 colonies of *Sterigmatomyces acheniorum* (CBS 6386) growing on methyl α -D-glucoside as sole source of carbon with conditions as for Fig. 4.

Fig. 6. Growth of *Sterigmatomyces acheniorum* (CBS 6385) on 50 mM-methyl α -D-glucoside (—), or D-glucose (-----), for a subculture derived from O, a large colony; and for ●, a small colony (see text). Conditions were as for Fig. 3.

DISCUSSION

The strains of yeast described here did not appear to form ascospores, were non-filamentous and produced daughter-cells on sterigmata. Together, these three features are characteristic of *Sterigmatomyces* Fell (Fell, 1970) and of no other genus of yeast (Lodder, 1970). Had the colonies been notably pink from carotenoid pigment, these yeasts might have been considered as *Rhodotorulae*, though budding on sterigmata is not a feature of that genus (Phaff & Ahearn, 1970). Verona & Rambelli (1962) described features of some species of *Taphrina* that are common to *Sterigmatomyces acheniorum*, notably the sterigmata. However, unlike the latter, *Taphrina* species do not utilize either lactose or erythritol (Mix, 1954). Furthermore, they form starch which, according to Lodder (1970), is taxonomically critical in the present context.

Among the existing species of *Sterigmatomyces*, *Sterigmatomyces acheniorum* most closely resembled the description of *S. halophilus*, but differed markedly from the latter in utilizing readily sucrose, maltose, raffinose, melezitose and also in being non-halophilic. Because of these differences, we gave our strains a new specific name, 'acheniorum', as they were associated with the achenes and receptacle of *Fragaria ananassa*.

The slow growth on certain glycosides was examined as a special feature of the species. However, some incidental observations on other yeasts suggest that our findings may well apply quite widely in the yeasts and be of general interest. The repeated occurrence of such large and small colonies as those shown in Fig. 4 must have been interpreted from time to time in terms of mixed cultures in the sense of their not having been derived from a

single-cell isolation. Particular care was taken in purifying our strains to avoid such a mistake, though no physiological explanation is offered of our observations on the sizes of the colonies.

Three strains of *Sterigmatomyces acheniorum* have been deposited in the collection of the Centraalbureau voor Schimmelcultures, Delft; no. 6386 is the type strain.

Sterigmatomyces acheniorum species nova

In liquore definito cum D-glucoso, in augendo ad 25 °C, cellulae sunt in forma aut ellipses aut piri, 2.6 ad 7.9 × 1.6 ad 4.0 µm. Gemmae e sterigmatibus (circa 1 µm longorum) fere faciunt. Ascosporae, ballistosporae, hyphae, pseudohyphaeque non sunt. Non est fermentatio. Fermenta speciei huius crescunt in liquore definito cum raffinoso, melezitoso, sucroso, maltoso, trehaloso, methyl α-D-glucosido (lente), cellobioso (lente), melibioso (lente), lactoso (lente), D-glucoso, D-galactoso, L-sorboso (lente), D-riboso, D-xyloso, D-arabinoso (lente), L-arabinoso, succinato, citrato, lactato (lente), glycerolo (lente), erythritolo, ribitolo (lente), D-glucitolo, D-mannitolo, inulino (lente), nitrato. Non crescunt cum salicino, L-rhamnoso, galactitolo, myo-inositolo, amylo. Vitaminas addere necessarium est. In 37 °C non crescunt; in 30 °C lente crescunt. In 50 % D-glucoso non crescunt. Amylum non formant. Urea hydrolysantur.

Typus cultura 6386 in collectione Centraalbureau voor Schimmelcultures, Delft, isolata e fragoribus.

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