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Zygozyma smithiae sp.n. (Lipomycetaceae), a new ambrosia yeast from Southern Africa

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Abstract

A new species of the genus *Zygozyma, Z. smithiae,* was recovered from frass of the ambrosia beetle, *Crossotarsus externedentatus* in Northern Natal. A description of the new species and key to the genus are given.

Introduction

The genus *Zygozyma* Van der Walt & Von Arx (Van der Walt et al. 1987) is currently known from three species, *Z. oligophaga* Van der Walt & Von Arx (Van der Walt et al. 1987), *Z. suomiensis* Smith et al. (1989), and *Z. arxii* Van der Walt et al. (1990). In the present communication a fourth, entomophorous species, recovered from insect frass, is described.

Material and methods

The two strains studied, CBS 7407 and CBS 7408, are held by the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft (The Netherlands). Their morphological and physiological characters were determined by the conventional methods described by Van der Walt & Yarrow (1984). The utilization of carbon sources was examined at 25° C on a Tissue Culture Rollordrum (New Brunswick) operating 40 rph. Results were scored after 3, 7 and 14 days. Complete utilization of a carbon source occurring only after 7 days was scored as delayed (D). The utilization of nitrogen sources was examined by the conventional auxanographic technique over a period of 10 days. The ubiquinone isoprenologues of the type strain were determined according to the method of Yamada & Kondô (1971, 1973). The extraction and purification of the nDNA of the strains were performed as described by Smith & Poot (1985). The base composition of the nDNA was determined in 0.1 SSC (SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0 ± 0.2) from the thermal denaturation profile according to the method of Owen et al. (1969) and calculated from the formula Mol% G + C = 2.08 Tm − 106.4. A standard preparation of nDNA of *Candida parapsilosis* CBS 604 (Tm = 70.6° C in 0.1 SSC) was included in every determination as control. The values reported reflect the mean and standard deviation of three determinations. Observations by TEM were based on ascigerous material of the type strain from a 6 day-old culture grown at 18° C on 2% malt agar. Material was fixed in 1.5% aqueous potassium permanganate as described by Van der Walt et al. (1983).

Description

Zygozyma smithiae Van der Walt, Wingfield & Yamada sp.n. (Lipomycetaceae)

In extracto malti post triduum 25° C cellulae incapsulatae, aut globosae ellipsoideaeque 3.0–7.0 μm singulae binaeque, aut globosae ovoideae ellipsoideae botuliformes vel cylindratae interdum basibus latis 5.0–10.0 × 4.5–9.0 μm aggregatae. Asci immaturi adsint. Incrementum exiguum. Post hebdomades 4 temperatura ambeunte annulus inchoatus et sedimentum plus minusve floccosum adsunt. In agaro malti post triduum 25° C cellulae incapsulatae, aut globosae ellipsoideaeque 2.0–6.0 μm singulae binaeque, aut globosae ellipsoideae botuliformes vel cylindratae amoeboideae interdum basibus latis 4.0–9.0(–15.0) × 4.0–7.0 μm aggregate. Asci immaturi adsint. Cultura aquosa vel mucosa, hyalina vel creme-opacca, glabra nitida margine glabro. Post hebdomades 4 temperatura ambeunte hyalina vel bruneoli-cremea glabra vel crispulata nitida. Margo integer. In agaro farinae Zeae maydis confecto post decemduum 25° C pseudohyphae et hyphae nullae. Asci non juncti affixi globosi allantoideae amoeiboidei multispori evanescentes, 9.0–18.0 × 3.0–9.0 μm, oriundi intercalariter vel terminaliter per transformationem directas cellularum singularum vegetivarum aggregatarum. Ascosporae allantoideae vel cymbiformes, 2.5–3.5 × 1.0–1.5 μm, glabrae succinae conglutinantes ubi liberatae. Fermentatio gaseosa glucosi nulla. D-xyloso(lente) sucrose glycerolo (lente) xylitolo D-glucono-1,5-lactono 2-keto-D-gluconato 5-keto-D-gluconato(lente) quinato succinato ethanolo(lente) propano-1,2-diolo(lente) ethylamino hydrocholorico cadaverino dihydrochlorico L-lysino creatinino(lente) et imidazolo utitur nec D-galactoso L-sorboso D-glucosamino D-riboso L-arabinoso D-arabinoso L-rhamnoso maltoso α,α-trehaloso α-methyl-D-glucosido cellobioso salicino melibioso lactoso raffinoso melezitoso inulino amylo solubili *m*-erythritolo ribitolo L-arabitolo, D-glucitolo D-mannitolo galactitolo *m*-inositolo D-gluconato D-glucuronato DL-lactato citrato methanolo butano-2,3-diolo nitrato nitrito nec creatino. Non crescit nisi vitamina necessaria dantur. Crescit coram parte una cycloheximidi per mille. Non crescit in agaro extracto fermenti confecto quinquaginta partes glucosi per centum a pondere continente. Non crescit in 37° C. Materia amyloidea formatur. Ureum non finditur. Ubiquinonum majus Q9. G + C acidi deoxyribonucleati 55.7 ± 0.3 mol per centum (CBS 7407T).

Growth in malt extract

After 3 days at 25° C the encapsulated vegetative cells are either globose to ellipsoid, 3.0–7.0 μm, occurring singly or in pairs, or globose, ovoid, ellipsoid, botuliform to short cylindrical, occasionally with broad bases, 5.0–10.0 × 4.5–9.0 μm, occurring in aggregates. Immature asci may be present. Growth is scant. After 4 weeks at room temperature an incomplete ring and a more or less floccose sediment are present.

Growth on malt agar

After 3 days at 25° C the encapsulated vegetative cells are either globose to ellipsoid, 2.0–7.0 × 2.0–6.0 μm, occurring singly or in pairs, or globose, ellipsoid, botuliform to short cylindrical occasionally with broad bases, 4.0–9.0(–15.0) × 4.0–7.0 μm, occurring in aggregates. Immature asci may be present. The streak culture is watery to mucoid, hyaline to creamish opaque, smooth, shiny with an entire margin. After 4 weeks at room temperature the culture is hyaline to brownish-cream, smooth to crispulated and shiny. The margin is entire.

Dalmau plate cultures on corn meal agar

After 10 days at 25° C neither hyphae nor pseudohyphae are formed.

Formation of ascospores

Asci are unconjugated, attached, globose allantoid or amoeboid, multispored, 9.0–18.0 × 3.0–9.0 μm,

Utilization of carbon sources			
D-Galactose	–	Ribitol	–
L-Sorbose	–	Xylitol	D
D-Glucosamine	–	L-Arabitol	–
D-Ribose	–	D-Glucitol	–
D-Xylose	D	D-Mannitol	–
L-Arabinose	–	Galactitol	–
D-Arabinose	–	*m*-Inositol	–
L-Rhamnose	–	Glucono-δ-lactone	D
Sucrose	+	2-Keto-D-gluconate	+
Maltose	–	5-Keto-D-gluconate	D
ααTrehalose	–	D-Gluconate	–
αMe-D-glucoside	–	D-Glucuronate	–
Cellobiose	–	D-Galacturonate	–
Salicin	–	DL-Lactate	–
Melibiose	–	Succinate	+
Lactose	–	Citrate	–
Raffinose	–	Methanol	–
Melezitose	–	Ethanol	+
Inulin	–	Propane 1,2 diol	D
Sol. starch	–	Butane 2,3 diol	–
Glycerol	D	Quinate	+
m-Erythritol	–	Phloroglucinol	–

Utilization of nitrogen sources			
Nitrate	–	Creatine	–
Nitrite	–	Creatinine	D
Ethylamine hydrochloride	+	Glucosamine	–
Cadaverine dihydrochloride	+	Imidazole	+
L-Lysine	+		

Splitting of Arbutin	: Absent
Growth in vitamin-free medium	: Absent
Growth with 0.1% cycloheximide	: Positive
Growth on 50% (m/m) glucose	
Yeast extract agar	: Absent
Formation of amyloid	: Positive, colouring blue-green with iodine
Splitting of urea	: Absent, or very weak
Major ubiquinone system	: Q9 (CBS 7407T)
Mol % G + C	: 55.7 ± 0.3 (CBS 7407T), 55.3 ± 0.4 (CBS 7408)

evanescent, arising by the direct transformation of presumed diploid, aggregated, vegetative cells. The mature ascospores are allantoid to cymbiform, 2.5–3.5 × 1.0–1.5 μm, glabrous (Fig. 1), amber-coloured, and conglutinated when liberated. Ascus

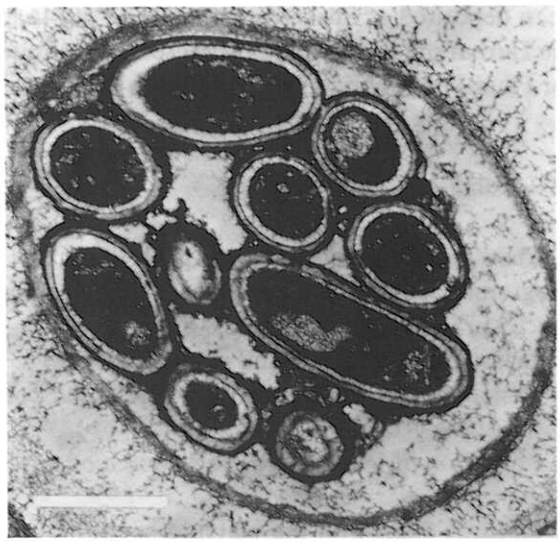

Fig. 1. Zygozyma smithiae CBS 7407. TEM micrograph of an ascus showing allantoid ascospores in longitudinal and transverse section. Insert bar = 1 μm. (Material fixed in 1.5% aqueous potassium permanganate).

formation was observed on 2% malt agar after 6 days at room temperature.

Etymology

Smithiae, L. gen. (f) of Smith, the species being named for Dr Maudy Th. Smith, in recognition of her merritorious contributions in yeast systematics.

Origin of strains studied

The two strains studied were isolated from two frass samples of the platypid ambrosia beetle, *Crossotarsus externedentatus* (Fairm.) Scheidl, infesting a moribund specimen of *Macaranga capensis* (Baillon) Benth. in the State Forest at Kwa-Mbonambi (Northern Natal) South Africa. (From the same habitat a second strain of *Z. oligophaga*, CBS 7406, was simultaneously recovered.) The two strains have been deposited in the Yeast Collection of the Centraalbureau voor Schimmelcultures in Delft (The Netherlands) as CBS 7407 (type) and CBS 7408. The non-living holotype has

been deposited as Specimen No. PREM 50444 in the Herbarium for Fungi of the Research Institute for Plant Protection in Pretoria (South Africa).

Discussion

The new species is assigned to the genus *Zygozyma* on grounds of its close agreement with the type species, *Z. oligophaga,* in terms of:

- ascosporal shape, size and pigmentation,
- formation of extracellular amyloid which colours blue-green with iodine,
- utilization of imidazole as sole source of nitrogen, and
- an association with the platypid beetle, *C. externedentatus*.

The two species consequently appear to be sympatric and representative of the, so-called, ambrosia yeasts, which underlines the wide, natural distribution of the Lipomycetaceae. *Zygozyma smithiae,* like *Z. arxii,* nevertheless, differs from *Z. oligophaga* by the Coenzyme Q9 system. *Zygozyma smithiae* is distinguished from all other described members of the genus by the unusually high guanine + cytosine content of its nDNA (55 mol%) – a feature previously thought to be characteristic of basidiomycetous taxa (Meyer & Phaff 1970).

The four species currently assigned to the genus may conveniently be recognized by the following:

Key to the genus *Zygozyma*

1.	Sucrose utilized	*Z. smithiae*
	Sucrose not utilized	2
2.	Trehalose utilized	*Z. arxii*
	Trehalose not utilized	3
3.	L-Arabinose utilized	*Z. suomiensis*
	L-Arabininose not utilized	*Z. oligophaga*

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