PICHIA KODAMAE SP. NOV., A NEW HOMOTHALLIC YEAST SPECIES

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Four strains, representative of an undescribed species of *Pichia*, have been recovered from insect infestations of *Euphorbia ingens* in South Africa. A description of the new species, *Pichia kodamae*, is given. The type strain of *Pichia kodamae* was found not to hybridize with strains of *Pichia pini*, *Pichia methanolica* and *Pichia cellobiosa*.

During the course of the study of yeasts associated with insect infestations of *Euphorbia ingens* E. Mey., four strains of an undescribed, homothallic *Pichia* species were recovered. Although the new species appears to be close to *Pichia pini* (Holst) Phaff, *Pichia methanolica* Makiguchi and *Pichia cellobiosa* Lee et Komagata, it is nevertheless readily differentiated from these three taxa on the basis of the apparent absence of hybrid formation, the inability to utilize methanol and the slow utilization of galactitol in the absence of galactose utilization.

MATERIALS AND METHODS

Strains. The following prototrophic strains are held by the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, (The Netherlands): CBS 7081; isolated from *Euphorbia ingens* (January, 1980)

Pichia cellobiosa CBS 8002

Pichia methanolica CBS 6515

Pichia pini CBS 2613

Auxotrophic mutants

CBS 7081 *ade*

CBS 7081 *arg*

CBS 7081 *cys*

CBS 7081 *lys*

Pichia methanolica

CBS 6515 *arg*

Pichia pini

CBS 2613 *ade*

CBS 7081 *ura*
Pichia cellobiosa
CBS 8002 *ade*

CBS 2613 *arg*
CBS 2613 *gua*
CBS 2613 *ura*

The notations *ade*, *arg*, *cys*, *gua*, *his*, *lys*, and *ura* denote requirements for adenine, arginine, cysteine, guanine, histidine, lysine and uracil, respectively.

Media. 1. Yeast extract-malt extract (YM) agar (1). 2. The minimal recovery agar (MRA) medium consisted of WICKERHAM's (1) nitrogen base with the omission of amino acids, but supplemented with 1.5% washed agar and 1.0% w/v of the filter-sterilized carbohydrate source selected for the recovery of specific recombinants.

Mutant induction and characterization. Auxotrophic mutants were induced with N-methyl-N'-nitro-N-nitrosoguanidine at final concentrations of 500 µg/ml at pH 5.0 and pH 8.0 according to the technique of MORI (2) and DEL GIUDICE and PUGLISI (3). Isolation, selection and the characterization of the mutants were carried out as previously described by VAN DER WALT and JOHANNSEN (4).

Hybridization technique and recovery of hybrids. Parental strains were mass-mated on YM agar slants and incubated at 28° for 7 days. After confirming ascospore formation, the mated cultures were serially transferred onto fresh YM agar slants at 7-day intervals over a period of 4 weeks. After confirming ascospore formation in the fourth transfer of the mated cultures, the growth was taken up in 2.0 ml of sterile tap water, washed, resuspended in the same volume of sterile tap water, and enumerated by serial dilution plate technique for the recombinant count on MRA containing a carbon source which permitted the development of expected recombinants only. Colonies on YM agar plates were enumerated after 3 days and on MRA plates after 7 and 14 days. For further experimental details concerning the handling of control cultures and the detection of back-mutation VAN DER WALT and JOHANNSEN (4) must be consulted. The results of the crosses made are shown in Tables 1-4.

Transmission electron microscopy (TEM). TEM of ultra-thin sections of the ascospores of 4 day-old cultures on Difco malt agar of CBS 7081, fixed in 1.5% aqueous potassium permanganate, is based on the method described by KREGER-VAN RIJ and VEENHUIS (5).

The description of the new species is based on the standard methods adopted for yeast taxonomy (6).

RESULT AND DISCUSSION

The recovery of prototrophic recombinants from intrastrain crosses of CBS 7081 as shown in Table 1, confirms the applicability of the prototrophic selection technique for the detection of hybrid formation in *Pichia* species characterized by life-cycles which implicate the fusion of independent, free-occurring, haploid cells.

Table 1. Intrastrain crosses of auxotrophic mutants of CBS 7081 in which prototrophic recombinants were detected on minimal glucose agar.

Parental strains	Viable count ($\times 10^7$ /ml)	Recombinants/ 10^7 cells of the mated population
CBS 7081 <i>ade</i> $\times$ CBS 7081 <i>cys</i>	1.2	10.1×10^4
CBS 7081 <i>arg</i> $\times$ CBS 7081 <i>ura</i>	8.0	4.2×10^3

Table 2. Crosses between CBS 7081 and auxotrophic mutants of *Pichia pini* CBS 2613 in which recombinants were not detected.

Parental strains	Carbon source in recovery medium	Viable count in which recombinants were not detected ($\times 10^7$)
CBS 7081 <i>tre</i> $\times$ CBS 2613 <i>ade</i> TRE ^a	trehalose	40
CBS 7081 <i>tre</i> $\times$ CBS 2613 <i>arg</i> TRE	trehalose	24
CBS 7081 <i>tre</i> $\times$ CBS 2613 <i>gua</i> TRE	trehalose	23
CBS 7081 <i>tre</i> $\times$ CBS 2613 <i>ura</i> TRE	trehalose	21

^a The notation TRE denotes the oxidative utilization of trehalose and *tre*, the failure to utilize this sugar.

Table 3. Crosses between auxotrophic mutants of CBS 7081 and *Pichia methanolica* CBS 6515 in which recombinants were not detected.

Parental strains	Carbon source in recovery medium	Viable count in which recombinants were not detected ($\times 10^7$)
CBS 7081 <i>ade</i> $\times$ CBS 6515 <i>arg</i>	glucose	4.3
CBS 7081 <i>his</i> $\times$ CBS 6515 <i>arg</i>	glucose	3.9
CBS 7081 <i>lys</i> $\times$ CBS 6515 <i>arg</i>	glucose	1.6
CBS 7081 <i>ura</i> $\times$ CBS 6515 <i>arg</i>	glucose	3.7

Table 4. Crosses between auxotrophic mutants of CBS 7081 and *Pichia cellobiosa* CBS 8002 in which recombinants were not detected.

Parental strains	Carbon source in recovery medium	Viable count in which recombinants were not detected ($\times 10^7$)
CBS 7081 <i>arg</i> $\times$ CBS 8002 <i>ade</i>	glucose	8.3
CBS 7081 <i>his</i> $\times$ CBS 8002 <i>ade</i>	glucose	1.7
CBS 7081 <i>arg</i> $\times$ CBS 8002 <i>ade</i>	glucose	1.6
CBS 7081 <i>his</i> $\times$ CBS 8002 <i>ade</i>	glucose	0.8

The failure to recover prototrophic recombinants from the crosses shown in Tables 2, 3 and 4 suggest that the strain CBS 7081 does not hybridize with either *Pichia methanolica*, *Pichia pini* or *Pichia cellobiosa* and that it is genetically isolated from representatives of these three species. In view of this genetic isolation, strain CBS 7081 and the three phenotypically-identical strains are assigned to:

Pichia kodamae van der Walt et Yarrow sp. nov. (Endomycetaceae)

Species homothallica isolata instar cellularum diploidearum haploidearumque.

In extracto malti post dies 3 28° cellulae vegetativae globosae, subglobosae, ovoideae, ellipsoideae vel bacilliformes, $3.0\text{--}6.0 \times 2.5\text{--}4.5 \mu\text{m}$, incapsulatae, singulae vel binae vel catenis brevibus vel racemis parvis connexae. Post unum mensem temperatura ambeunte sedimentum et annulus tenuissimus adsunt.

In agaro malti post dies 3 28° cellulae eiusdem formae, $1.5\text{--}6.0 \times 1.5\text{--}4.5 \mu\text{m}$, incapsulatae, singulae vel raro catenis brevibus connexae. Culturae nitidae, glabrae, aliquantum cremeae, hyalinae, viscosae vel glutinosae, margine glabro. Culturae post unum mensem temperatura ambeunte nitidae, glabrae, glutinosae, margine glabro.

In agaro farina *Zea mays* confecto post dies 10 hyphae et pseudohyphae nullae.

Asci evanescentes e cellulis conjunctis an non-conjunctis oriundi, uni- ad quadrispora. Ascosporae maturae galeiformes, $2.0 \times 1.0 \mu\text{m}$ (ora inclusa), inter se agglutinatae.

Glucosum solum fermentatur.

Glucosum, L-sorbosum, cellobiosum, D-xylosum, L-arabinosum, D-arabinosum (lente), D-ribosum, ethanolum (infirmum), glycerolum, *i*-erythritolum, ribitolum, galactitolum (lente), D-glucitolum, D-mannitolum, salicinum, DL-acidum, lacticum (infirmum), acidum succinicum et acidum citricum assimilantur neque galactosum, sucrosus, maltosus, trehalosus, lactosus, melibiosus, raffinosis, melezitosis, inulinum, amyllum solubile, L-rhamnosus, methanolum, α -methyl-D-glucosidum nec *i*-inositolum.

Ethylaminum hydrochloricum assimilatur neque nitras kalii nec nitris natrii.

Vitamina externa ad crescentiam necessaria sunt.

Crescere potest 37°.

Typus cultura CBS 7081, isolatus e textu *Euphorbiae ingentis* a larvis Cerambycidarum infestatae in districto Groblersdalensi (Africa australis) lectae, in collectione zymotica Centraalbureau voor Schimmelcultures, Delphis Batavorum praeservatus.

Holotypus materia ascigera stirpis typicae permanganato kalii fixa, inclusa in Araldite-Epon permixtione, specimen no. PREM 45951 in herbario mycologico Instituti Investigationis Custodiaeque Plantarum, Praetoriae in Africa australi praeservatus.

Growth in malt extract: After 3 days at 28° the cells are spheroidal, subglobose, ovoid, ellipsoidal or bacilliform, $3.0\text{--}6.0 \times 2.5\text{--}4.5 \mu\text{m}$, encapsulated, and occur singly, in pairs or short chains or small clusters. A sediment and slight incomplete ring are formed. After 1 month at room temperature a sediment and slight ring are present.

Growth on malt agar: After 3 days at 28°, the cells are spheroidal, subglobose, ovoid, ellipsoidal to bacilliform, $1.5\text{--}6.0 \times 1.5\text{--}4.0 \mu\text{m}$, encapsulated and occur singly, in pairs, rarely in short chains. The streak culture is glistening,

smooth, creamish, hyaline, viscous to glutinous. The margin is entire. After 1 month at room temperature, the streak culture is shiny, smooth, creamish-grey and glutinous. The margin is entire.

Dalmau plate cultures on corn meal agar: Neither hyphae nor pseudohyphae are formed.

Ascospore formation: Evanescent, 1–4 spored asci arise either by the transformation of diploid cells or by the conjugation of haploid cells. Mature ascospores are galeate, $2.0 \times 1.0 \mu\text{m}$, including the brim, and on liberation, tend to agglutinate (Fig. 1). Ascospore formation is observed on YM agar and Difco malt agar.

Fermentation:

Glucose	+	(slow)			
Galactose	—		Maltose	—	
Sucrose	—		Lactose	—	
Assimilation of carbon sources:					
Glucose	+		D-Ribose	+	
Galactose	—		L-Rhamnose	—	
L-Sorbose	+		Methanol	—	
Sucrose	—		Ethanol	+	(weak)
Maltose	—		Glycerol	+	
Cellobiose	+		<i>i</i> -Erythritol	+	
Trehalose	—		Ribitol	+	
Lactose	—		Galactitol	+	(slow)
Melibiose	—		D-Glucitol	+	
Raffinose	—		D-Mannitol	+	
Melezitose	—		α -Methyl-D-glucoside	—	
Inulin	—		Salicin	+	
Soluble starch (Merck's)	—		DL-Lactic acid	+	(weak)
α -Xylose	+		Succinic acid	+	
L-Arabinose	+		Citric acid	+	
D-Arabinose	+	(slow)	<i>i</i> -Inositol	—	

Assimilation of nitrogen sources:

Potassium nitrate	—
Sodium nitrite	—
Ethylamine hydrochloride	+

Growth in vitamin-free medium: Absent.

Growth in 10% sodium chloride plus 5% in yeast nitrogen base: Absent.

Growth at 37°: Positive.

Strains studied.

Four strains were studied. These were recovered from decaying tissue infested by Cerambycid larvae, of specimens of *Euphorbia ingens* E. Mey., growing in the Groblersdal district (South Africa).

Fig. 1. *Pichia kodamae*. Electron micrograph of liberated ascospores. The insert bar represents 0.5 μ m.

Material fixed in 1.5% aqueous potassium permanganate, stained with uranyl acetate and Reynolds' lead citrate and embedded in an Araldite-Epon mixture.

Type strain of *Pichia kodamae* is maintained as culture No. 7081 in the Yeast Collection of the Centraalbureau voor Schimmelcultures. The holotype of *Pichia kodamae* is constituted by potassium permanganate-fixed, ascigerous material from the type strain embedded in an Araldite-Epon mixture. This non-living material has been deposited as Specimen No. 54951 in the Mycological Herbarium of the Research Institute for Plant Protection in Pretoria, South Africa.

Etymology: *kodamae* Lat. gen., of Kodama, the species being named in honour of Dr. Kenkichi Kodama in recognition of his contributions to yeast ecology and taxonomy.

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