

## *Williopsis salicorniae* Sp. Nov.

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### Abstract

Four strains of an undescribed species of the genus *Williopsis* were isolated from brackish water. A description of the new species, *Williopsis salicorniae* (type strain, CBS 8071, NRRL Y-12834), is given.

### Introduction

Four yeast strains were isolated from the brackish water (45–50 g/l NaCl) of a salt-spring situated near Château-Salins (North-East of France 'department Moselle' 57000) which also contained a wide variety of other yeasts (Hinzelin & Lectard 1985). The four isolated, which reproduce asexually by multi-lateral budding and sexually by forming asci (not borne on ascophores) with 1–4 hemispheroidal to Saturn-shaped ascospores fit the diagnosis of the genus *Williopsis* Zender (Kurtzman 1984). Since these strains could not be identified with any of the *Williopsis* species now known, we propose to describe them as a new species.

### Materials and methods

#### *Morphological and physiological characteristics*

The morphological and physiological characteristics for the description of the new taxon are based on the methods commonly applied in yeast taxonomy (van der Walt & Yarrow 1984). For growth tests

on carbon compounds, cultures were incubated on a rocking shaker at 30 cycles/min. for 28 days at 25°C. Growth on nitrogen sources was examined in auxanograms for 7 days.

#### *DNA Isolation and characterization*

DNA was isolated and purified according to the methods of Price et al. (1978). The DNA base composition of the type strain was determined from buoyant density in CsCl by using the equation of Schildkraut et al. (1962).

### Results and discussion

#### *Williopsis salicorniae*

Hinzelin, Kurtzman & M. Th. Smith, sp. nov.

In extracto malti post dies 3, 25°C cellulae globosae vel ovoideae, 3–8 × 3–6.5 µm undique gemmantes, singulae vel binae. Neque velum nec annulus formantur, sed sedimentum adest. In agarò farinae *Zea mays* confecto pseudomycelium nullum. Cultura in agarò peptono cum glucoso et extracto

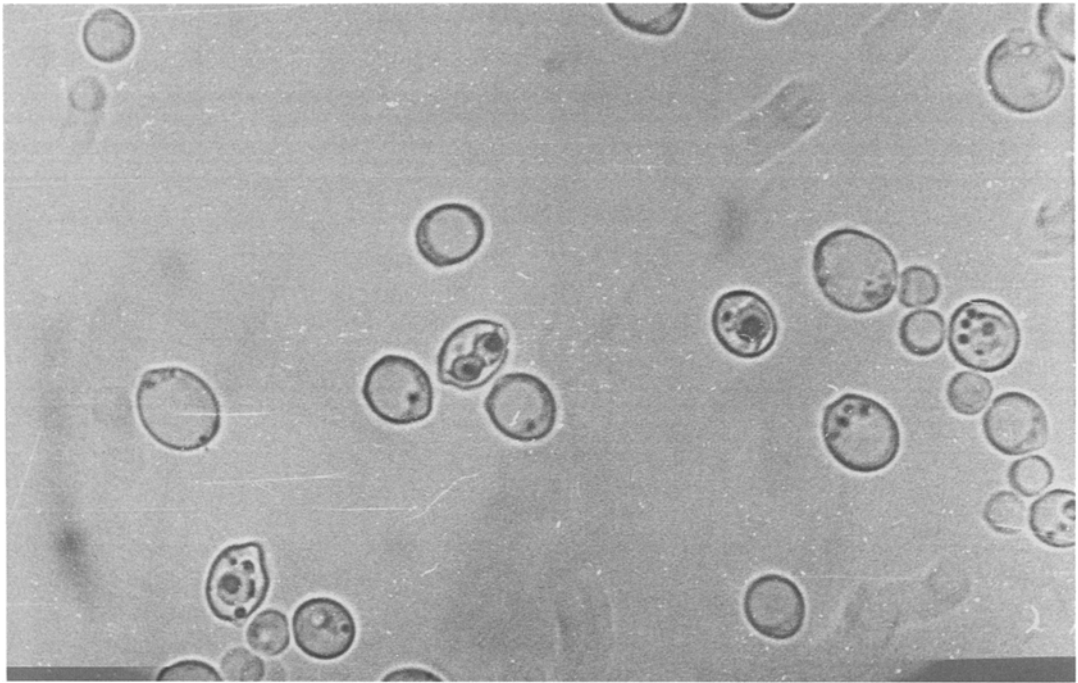


Fig. 1. Asci containing globose to ellipsoidal ascospores with a small ledge. On Difco YM agar, 15–21 days. 1500 ×

levedinis post hebdomades 4 temperatura ambeunte, eburnea, glabra, margine integro. Asci continentens (1–)2(–4) sporas. Ascosporae maturae globosae vel ellipsoidae muro leviter rugoso et indistincta ora  $2.5\text{--}5 \times 2\text{--}3.5 \mu\text{m}$ . Fermentatio et crescentia in variis substratis carbonis et nitrogeni et reliqui chatacteres in tabula 1 monstrantur. Typus CBS 8071, isolatus ex aqua salina exsiccatus in CBS, Baarn, et vivus in collectione zymotica Delphis Batavorum praeservatur.

#### *Growth in malt extract*

After 3 days at 25° C, the cells are globose to subglobose, very rarely ellipsoid or ovoid  $3\text{--}8 \times 3\text{--}6.5 \mu\text{m}$ ; they are single, in pairs, or in small clusters. Vegetative reproduction occurs by multilateral budding. Neither a pellicle nor a ring is formed; a light sediment is present.

#### *Growth on malt agar*

After 3 days at 25° C, the cells are globose to subglobose, rarely ovoid or ellipsoid,  $4\text{--}10 \times 3\text{--}9 \mu\text{m}$ , and occur singly or in pairs. After 3 weeks the

streak culture is creamcoloured and with an entire or slightly lobed margin; the surface is smooth and dull, the texture is soft.

#### *Dalmau plate cultures on corn meal agar*

Pseudomycelium is not formed.

#### *Formation of asci and ascospores*

Sporulation was observed on YM agar and corn meal agar. Vegetative cells are transformed into unconjugated, persistent asci. Asci  $6\text{--}9 \times 5\text{--}8 \mu\text{m}$ , are 1–4–, usually 2-spored. Ascospores are globose to ellipsoid,  $2.5\text{--}5 \times 2\text{--}3.3 \mu\text{m}$ , and have a thin equatorial ledge that can usually be seen with the light microscope. Occasionally, surfaces of some ascospores appear faintly roughened (Fig. 1).

#### *Physiological characteristics*

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

*Type strain*

CBS 8071, isolated from brackish water in a salt-spring, was deposited as a living culture in the Centraalbureau voor Schimmelcultures, Delft, and as a dried specimen at CBS, Baarn.

*Williopsis salicorniae* is unique among the saturn-

Table 1. Physiological characteristics of *Williopsis salicorniae* sp. nov.

<i>Fermentation</i>					
D-glucose	+	Sucrose	-	Raffinose	-
D-galactose	-	Trehalose	-	Xylose	-
Maltose	-	Lactose	-		
<i>Growth</i>					
D-glucose	+	Cellobiose	-	L-arabinitol	+
D-galactose	-	Salicin	-	D-glucitol	+
L-sorbose	-	Arbutin	-	D-mannitol	+
D-glucosamine	-	Melibiose	-	Galactitol	-
D-ribose	+	Lactose	-	Myo-inositol	-
D-xylose	+	Raffinose	-	D-glucono- $\delta$ -lactone	-
L-arabinose	+	Melezitose	-	2-keto-D-gluconate	-
D-arabinose	+	Inulin	-	D-gluconate	-
L-rhamnose	-	Soluble starch	-	DL-lactate	-
Sucrose	-	Glycerol	+	Succinate	-
Maltose	-	Erythritol	+	Citrate	-
$\alpha$ - $\alpha$ -trehalose	-	Ribitol	+	Methanol	-
$\alpha$ -methyl-D-glucoside	-	Xylitol	+	Ethanol	+
Nitrate	-	Vitamin-free medium	-		
Nitrite	-	30° C	-		
Ethylamine	+	37° C	-		
L-lysine	+	0,01 %	+		
		Cycloheximide			
Cadaverine	+	0,1 %	+		
		Cycloheximide			
Creatine	-	50 %	v		
		D-glucose			
Creatinine	-				
<i>Additional characteristics</i>					
Urease			-		
Starch formation			-		
Acetic acid production on Custer's medium			-		
G + C (CBS 8071 = NRRL Y-12834)			36,7 $\pm$ 0,11 %		
v = variable					

spored yeasts which are assigned to the genera *Williopsis* and *Pichia*. In particular, *W. salicorniae* assimilates L-arabinose and D-ribose, compounds not utilized by the other species.

Relationships among the saturn-spored yeasts were examined by Liu & Kurtzman (in preparation) from comparisons of ribosomal RNA sequences. The species formed two phylogenetic clusters that were quite distant from one another. One cluster was comprised of *W. saturnus* (Klöcker) Zender, *W. californica* (Lodder) von Arx, *W. pratensis* Babjeva & Reshetova, *W. salicorniae* and *P. mucosa* Wickerham & Kurtzman while the second cluster included *P. dispersa* (Dekker) Kreger-van Rij, *P. saitoi* Kodama et al., *P. zaruensis* Nakase & Komagata and *Pichia* sp. nov. These data led Liu & Kurtzman to conclude that the latter four species should be removed from *Pichia* and placed in a new genus rather than being assigned to *Williopsis*. The comparisons also showed that although *W. salicorniae* and the species presently designated as *P. mucosa* do not assimilate nitrate as a sole source of nitrogen, they nonetheless are relatively closely related to nitrate-assimilating species. Consequently, because of the ribosomal RNA comparisons, we have placed our new species in the genus *Williopsis*.

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