Saccharomyces inusitatus sp.n.

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The type strain of *Saccharomyces pastorianus* was found to disagree with the standard description given for this species by Lodder and Kreger-van Rij (1952). It ferments raffinose $\frac{1}{3}$ and fails to utilize melibiose and was consequently identified as *S. bayanus*.

Two yeast strains recovered from beer were, however, found to fit the description which Lodder and Kreger-van Rij (1952) gave for *S. pastorianus*. As *S. pastorianus* is a synonym of *S. bayanus*, these two strains have been assigned to a new species for which the name *S. inusitatus* is proposed.

INTRODUCTION

According to Lodder and Kreger-van Rij (1952), the name *Saccharomyces pastorianus* was first used by Reess (1870) and therefore preceded the advent of pure culture studies. Pasteur also employed this name in his "Etudes sur la bière" (1876). Hansen (1883, 1886, 1888), during his studies on the yeasts associated with brewing, established three types, viz. I, II and III, for the species. In 1904, however, he designated Type I as the true *S. pastorianus*. Types II and III he identified as *S. intermedius* and *S. validus* respectively. *S. pastorianus* had been isolated from the dust of a brewery in Copenhagen as well as from diseased beer. In his description of *S. pastorianus* Hansen (1904) reported that the species fermented glucose, sucrose, and maltose but not lactose. He did not study its capacity to ferment galactose or raffinose.

Stelling-Dekker (1931) retained *S. pastorianus* as a valid species but did not study Hansen's original strain. The strain examined by her was received by the Yeast Division of the Centraalbureau voor Schimmelcultures in 1930 from the National Collection of Type Cultures maintained by the Lister Institute of Preventive Medicine in London. All that is known of this strain, which was labelled *S. pastorianus*, was that it had been received from Klöcker. Stelling-Dekker reported that this strain fermented glucose, galactose, sucrose and maltose, and also fermented raffinose completely.

Lodder and Kreger-van Rij (1952) examined the original strain which Hansen had designated as *S. pastorianus* (i.e. *Saccharomyces pastorianus* Type I). They reported that it fermented glucose, sucrose and maltose. Galactose was not assimilated but raffinose was two thirds fermented, the galactose moiety being left intact. This unusual fermentation of raffinose distinguished *S. pastorianus* from *S. bayanus*. They also identified as *S. pastorianus* a second yeast strain which had been isolated by Lindner and had been obtained from Saaz as *S. cerevisiae*.

Windisch (1961) undertook a critical study of twenty strains labelled *S. pastorianus*, which included Hansen's type strain as well as the strain received from Saaz, and reported that none of these in fact fermented raffinose for two thirds. Both Hansen's and Saaz's strains as well as ten others fermented raffinose for one third but failed to ferment or assimilate galactose. The remaining eight strains fermented galactose and raffinose completely.

During the re-examination of cultures held by the Yeast Division of the Centraalbureau voor Schimmelcultures, both Hansen's type strain (CBS-538) and Saaz's strain (CBS-1174) were again investigated in some detail. It was confirmed that both strains failed to utilize galactose and fermented raffinose only for one third. Neither of the strains assimilated melibiose, which ability must be considered a *sine qua non* for two thirds fermentation of raffinose.

Since Hansen's type strain for *S. pastorianus* fits the description of *S. bayanus*, these two names refer to a single species. For the purpose of assigning priority to one of these names the following considerations must be taken into account. According to Lodder and Kreger-van Rij (1952) the name *S. bayanus* was introduced by Saccardo in 1895, while Hansen first used the name *S. pastorianus* in 1883. It was only in 1904 that it became possible to attach any specific significance to the name *S. pastorianus*, when Hansen designated a strain of his *S. pastorianus* Type I as the true *S. pastorianus*. In view of these considerations priority has been given to the name *S. bayanus*, so that *S. pastorianus* passes into synonomy in accordance with Article 57 of the International Code of Botanical Nomenclature accepted by the IXth International Botanical Congress.

During the re-investigation of the strains of the genus *Saccharomyces*, two strains, CBS-1546 and CBS-5148, both recovered from beer were encountered which actually fitted the description of *S. pastorianus* given by Lodder and Kreger-van Rij (1952). Neither assimilated galactose, but both fermented raffinose and melibiose, the latter very slowly (7 – 14 days). Raffinose was 2/3 fermented, both strains failing to ferment the galactose moiety. The presence of the unattacked galactose was detected as follows. The strains were allowed to ferment a 4% solution of raffinose in dilute yeast extract (1 : 10) in the custom-

ary Durham tubes until no further gas production was observed (17 days). The Durham tubes were then autoclaved at 10 lbs over-pressure for 15 minutes. After cooling, the tubes were inoculated with *S. unisporus*, CBS-398, which ferments galactose but not sucrose, trehalose, melibiose or raffinose. Gas production due to galactose fermentation was observed in the re-inoculated tubes within 24 hours. In the case of Hansen's type strain of *S. pastorianus*, which served as control, no residual galactose could be detected by this method.

As the nomenclatural type strain of *S. pastorianus* has proved to be identical with *S. bayanus*, the specific epithet *pastorianus* cannot be employed to designate these strains. The strains have therefore been assigned to a new species for which the name *Saccharomyces inusitatus* is proposed. This specific epithet has been chosen because of the species' unusual ability to ferment raffinose for two-thirds.

LATIN DIAGNOSIS

Saccharomyces inusitatus sp.n.

In extracto malti cellulae rotundae, ovoideae, cylindricae, elongataeque, (2.5 – 7.0) × (7.0 – 14.5)μ, singulae, binae, in catenis aut in racemis parvis. Sedimentum et aliquando annulus formantur.

In agar malti cellulae ovoideae, cylindricae, elongataeque, (2.7 – 7.5) × (5.5 – 15.0)μ, singulae, binae, in catenis aut in racemis parvis. Cultura conservata in aere cubiculi per unum mensem butyrosa, albiflava, glabra, verruculosa aut crispulata, parum acclivis, parum nitens. Margine glabro, lobiformo aut piloso.

Pseudomycelium.

Oriuntur asci per transformationem cellularum vegetarum diploidearum. Ascosporae glabrae, rotundae aut ovoideae; 1 – 4 in asco.

Glucosum, sucrosum, maltosum, raffinosum (bes), melibiosum (lente), melezitosum (var.), trehalosum (lente) et α-methyl-D-glucosidum (lente) fermentantur at non galactosum, lactosum, cellobiosum, inulinum, nec amylum solubile.

Glucosum, sucrosum, maltosum, trehalosum, melibiosum (lente), raffinosum, melezitosum (var.), ethanolum, glycerolum, D-mannitolum (var.), D-sorbitolum (var.),α-methyl-D-glucosidum (lente), acidum lacticum (exiguum) assimilantur, at non galactosum, lactosum, L-sorbosum, cellobiosum, inulinum, amylum solubile, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, erythritolum, adonitolum, dulcitolum, salicinum, acidum succinicum, acidum citricum, kalium gluconicum nec *i*-inositolum.

Arbutinum non finditur.

Kalium nitricum et ethylaminum hydrochloricum non assimilantur.

Ad crescentiam vitaminae externae non necessariae sunt.

Isolata ex cerevisia.

DESCRIPTION

Growth in malt extract: After 3 days at 28 C the cells are spheroidal, ellipsoidal, pyriform, cylindrical to elongate, (2.5 – 7.0) × (7.0 – 14.5)μ, and occur singly, in pairs, short chains or small clusters. Elongate or more or less filamen-

tous cells which may measure 25μ in length, may also be present. A sediment is formed.

After 1 month at room temperature a sediment is present and occasionally an incomplete ring as well.

Growth on malt agar: After 3 days at 28 C the cells are ellipsoidal, pyriform, cylindrical to elongate, (2.5 – 7.5) × (5.5 – 15.0)μ, and occur singly, in pairs, short chains or small clusters. Filamentous cells which may measure up to 40μ in length, may be present. The streak culture is butyrous, creamish-white to cream-coloured, occasionally raised, smooth to crispulate or somewhat rugose, occasionally somewhat spreading, somewhat shiny to rather dull. The margin may be entire to lobiform, partly fringed with pseudomycelium.

After 1 month at room temperature the culture is cream-coloured, somewhat raised, smooth or verruculose to crispulate, somewhat shiny to rather dull. The margin may be entire, undulating or lobiform, usually fringed with pseudomycelium.

Dalmau plate cultures on corn meal agar: Pseudomycelium is produced. It may be rudimentary, but is usually ramified.

Sporulation: Diploid vegetative cells are directly transformed into asci. Asci contain 1 – 4 smooth, spheroidal to prolate ellipsoidal ascospores. Sporulation was observed on Wickerham's MY agar and on Fowell's acetate agar.

Fermentation:

Glucose	+	Melezitose	– or + (slow)
Galactose	–	Trehalose	+ (occasionally slow)
Sucrose	+	Cellobiose	–
Maltose	+	Inulin	–
Lactose	–	Soluble starch	–
Raffinose	+⅔	α-Methyl-D-glucoside	+ (slow)
Melibiose	+ (slow)		

Assimilation of carbon compounds:

Glucose	+	D-Ribose	–
Galactose	–	L-Rhamnose	–
Sucrose	+	Ethanol	+
Maltose	+	Glycerol	+
Lactose	–	Erythritol	–
L-Sorbose	–	Adonitol	–
Cellobiose	–	Dulcitol	–
Trehalose	+	D-Mannitol	+ or –
Melibiose	+ (slow)	D-Sorbitol	+ or –
Raffinose	+	α-Methyl-D-glucoside	+ (occasionally weak)
Melezitose	– or +	Salicin	–

Inulin	–	Lactic acid	+ (occasionally weak)
Soluble starch	–	Succinic acid	–
D-Xylose	–	Citric acid	–
L-Arabinose	–	Potassium gluconate	–
D-Arabinose	–	*i*-Inositol	–

Splitting of arbutin: Absent.

Assimilation of potassium nitrate: Absent.

Assimilation of ethylamine hydrochloride: Absent.

Growth in vitamin-free medium: Positive.

Growth on 50% (*w*/*w*) *glucose yeast extract agar*: Absent.

Growth on 60% (*w*/*w*) *glucose yeast extract agar*: Absent.

Growth at 37C: Absent.

Growth with 100 *ppm actidone in the standard medium*: Absent.

The strain CBS-1546 is designated as the type for the species.

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