Microbiology, Vol. 74, No. 4, 2005, pp. 459–462. Translated from Mikrobiologiya, Vol. 74, No. 4, 2005, pp. 533–536.
Original Russian Text Copyright © 2005 by Yurkov.

EXPERIMENTAL ARTICLES

First Isolation of the Yeast *Saccharomyces paradoxus* in Western Siberia

A. M. Yurkov

Soil Science Faculty, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia

Received September 6, 2004; in final form, December 16, 2004

Abstract—Two ascomycetous yeast strains have been isolated near Novosibirsk from oak exudate. The strains have been identified as *Saccharomyces paradoxus* Bachinskaya based on the results of biochemical tests. The conspecificity of the isolates with *S. paradoxus* was confirmed by electrophoretic karyotyping and restriction analysis of the ITS region of its rDNA. This first isolation of *S. paradoxus* in Siberia provides evidence for the continuity of its natural habitats.

Key words: Saccharomyces paradoxus, exudate, western Siberia, karyotyping, restriction analysis.

The *Saccharomyces* sensu stricto complex, which, it was recently proposed, should be restricted to the genus *Saccharomyces* [1], contains at least six biological sibling species: *Saccharomyces cerevisiae* Meyen ex Hansen, *S. paradoxus* Bachinskaya, *S. bayanus* Saccardo, *S. cariocanus* Naumov *et al.*, *S. kudriavzevii* Naumov *et al.*, and *S. mikatae* Naumov *et al.* Isolation of the strains of this complex is almost completely limited to industrial fermentation processes. Not much is known about their distribution in nature. Representatives of *Saccharomyces paradoxus* have been most frequently found in natural habitats. This species has a vast natural habitat and is represented by a number of divergent populations: European [2–4], Far Eastern [5], Hawaiian [6], and North American [7]. However, the *Saccharomyces cerevisiae* habitat is thought of as interrupted, since representatives of this species have never been isolated in Siberia, where only representatives of the close species *Saccharomyces cerevisiae* have been found.

During our investigations of yeast communities on plant substrates in Novosibirsk oblast [8], we isolated, from oak exudates, two strains that were identified as *S. paradoxus* according to the results of biochemical tests. Molecular biological investigations confirmed the affiliation of these isolates with the species *S. paradoxus.* Both strains demonstrated intense sporulation. The isolation of these yeast strains in western Siberia is of considerable interest. Earlier, only representatives of *S. cerevisiae* [9] had been isolated in this region. The joint occurrence of different *Saccharomyces* species was previously noted in Far East [5].

The strains were deposited with the Collection of the Soil Biology Department, Faculty of Soil Science, Moscow State University, and given the strain designations KBP-3828 and KBP-3829.

MATERIALS AND METHODS

Samples of pedunculate oak (*Quercus robur*) exudate were obtained in August 2003 from the oak planting territory of the Central Siberian Botanical Garden, Siberian Division, Russian Academy of Sciences (Novosibirsk). In total, we analyzed 12 oak exudates from 8 trees. The samples of oak exudates were taken together with bark pieces. Two strains were isolated from two exudate flows in different parts of one tree trunk. The isolation of yeasts was carried out in accordance with the enrichment culture method. The bark pieces were incubated in a liquid malt extract medium at room temperature for 2–4 days until the appearance of signs of intensive fermentation. Then, the culture was plated onto malt extract agar that had been acidified to pH 4–4.5 with 40% lactic acid so as to inhibit bacterial growth. The plates were incubated at room temperature for a week. The grown yeast colonies were isolated in pure cultures.

Identification of the isolates was performed, based on morphological and physiological properties, according to determination manuals [10, 11].

Isolation and amplification of DNA was performed using a standard method. The amplification of 5.8S rDNA and the internal transcriptional spacers ITS1 and ITS2 (5.8S-ITS fragment) was accomplished with the use of the primers pITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and pITS4 (5'-CCTCCGCTTATTGATATGC-3'). A PCR reaction was run in 30 μl of a reaction mixture that contained a PCR buffer, 20 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.25 mM dNTP, 0.30 μmol of each primer, 1.25 units of *Tag* polymerase (Sintol, Russia), and 20 ng of the analyzed genomic DNA. A Tertsik thermal cycler (DNK-tekhnologiya, Russia) was operated on the following regime: initial heat denaturation of native DNA at 94°C

0026-2617/05/7404-0459 © 2005 Pleiades Publishing, Inc.

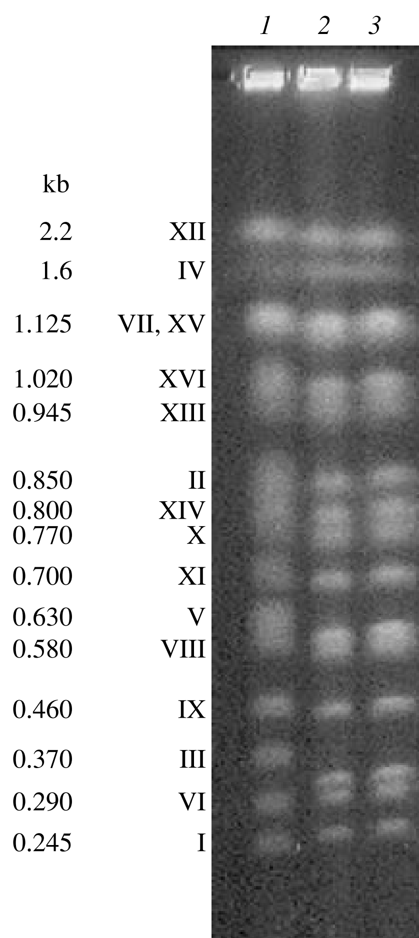

Fig. 1. Analysis of the chromosomal DNA of *Saccharomyces* strains by CHEF electrophoresis: (*1*) YNN-295 BioRad standard (BioRad, United States); (*2*) strain KBP-3828; (*3*) strain KBP-3829 .

for 3 min; then, 30 repetited cycles (DNA denaturation at 94°C for 2 min, primer annealing at 60°C for 1 min, and DNA synthesis at 72°C for 2.5 min); and final synthesis of the DNA at 72°C for 10 min. The amplified DNA was analyzed by electrophoresis at 60–65 V for 2 h in 1% agarose gel using 0.5× TBE buffer (45 mM Tris, 10 mM EDTA, and 45 mM Boric acid). The gel was stained with ethidium bromide.

Restriction fragment length polymorphism was analyzed using *Hpa*II and *Hae*III endonucleases (Fermentas, Lithuania). Separation of the restriction fragments was performed in 1.6% agarose gel in 0.5× TBE buffer at 60–65 V for 3 h. The gel was stained with ethidium bromide and photographed in ultraviolet light using a Vilber Lourmat transilluminator (France).

In preparation for electrophoretic karyotyping, the cultures were grown and chromosomal DNA was isolated according to the method described in [13]. Electrophoretic separation of chromosomal DNA was performed using a CHEF-DR^{TM}III apparatus for CHEF electrophoresis (Bio Rad, Richmond, CA, United States).

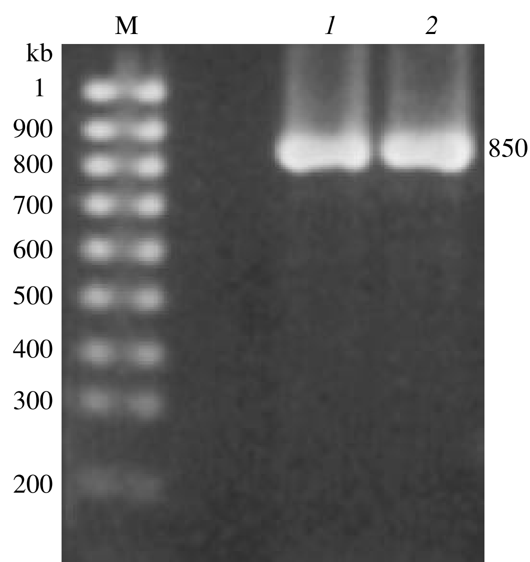

Fig. 2. Size of the 5.8S-ITS fragment in *Saccharomyces* strains (*1*) KBP-3828 and (*2*) KBP-3829. M indicates a 100-bp DNA Ladder (Fermentas, Lithuania) molecular weight marker (bp).

The electrophoresis was run in 0.5× TBE buffer at 200 V for 15 h with the electric field being switched every 60 s and then for 8 h with the electric field being switched every 90 s. Strain YNN-295(Bio Rad) was used as the standard.

RESULTS AND DISCUSSION

Phenotypic identification. Both isolates fermented glucose, galactose, and maltose and assimilated glucose, galactose, maltose, trehalose, raffinose, melezitose, ethanol, mannitol, and α-Me-D-glucoside as single carbon sources. The following compounds were not assimilated: sorbose, cellobiose, lactose, melibiose, inulin, starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, glycerol, erythritol, ribitol, dulcitol, D-sorbitol, salicin, succinate, citrate, inositol, 2-ketogluconate, and 5-ketogluconate. Nitrate was not used as the only source of nitrogen. Growth was possible up to 37°C. Intense sporulation was observed. The asci had a spherical shape and contained four oval spores. The combination of the above characteristics allowed the strains to be identified as representatives of the genus *Saccharomyces*.

Electrophoretic karyotyping. Yeasts of the genus *Saccharomyces* have a specific chromosome set, which can be visualized by pulsed-field electrophoresis (chromosomes are separated in an alternating electric field and then stained) [13, 14]. CHEF electrophoresis was used to compare the karyotypes of the new isolates with the *S. cerevisiae* karyotype. Both the new strains demonstrated a chromosomal set identical to the reference culture, *S. cerevisiae* YNN-295 (Bio Rad), for which the size of the chromosomes and their position after

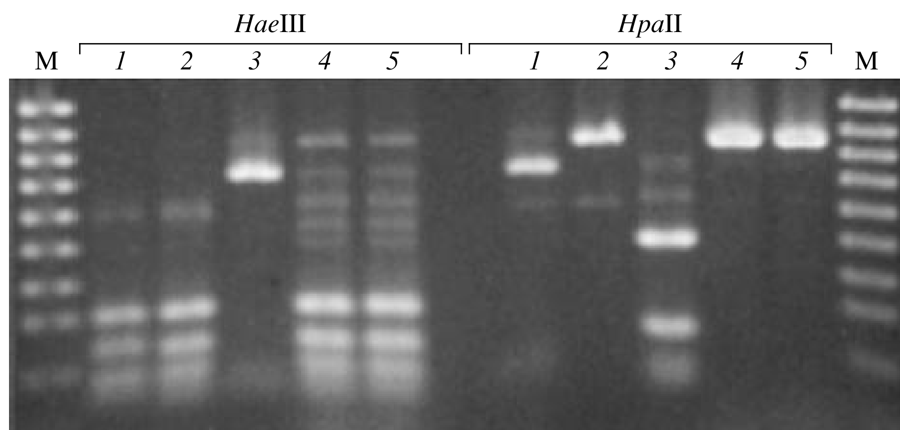

Fig. 3. Restriction analysis with the endonucleases *Hae*III and *Hpa*II of amplified 5.8S-ITS fragments of rDNA *Saccharomyces* strains: (*1*) reference culture *S. cerevisiae* CBS 1171; (*2*) reference culture *S. paradoxus* CBS 432; (*3*) reference culture *S. bayanus* CBS 380; (*4*) strain KBP-3828; (*5*) strain KBP-3829. M indicates a 100 bp DNA Ladder (Fermentas, Lithuania) molecular weight marker.

electrophoresis are known (Fig. 1). This analysis allowed us to strictly determine the affiliation of the isolates with the genus *Saccharomyces* [1]. In addition, among *Saccharomyces* species, only *S. cerevisiae* and *S. paradoxus* have an identical chromosome set, which allowed the taxonomic position of the analyzed strains to be determined more exactly.

Molecular differentiation. Earlier studies revealed that the six sibling species of the genus *Saccharomyces* differ in relation to their nucleotide sequences of rDNA ITS1 and ITS2 internal transcribed spacers [15] and can be differentiated by restriction analysis of these DNA fragments [12]. *S. cerevisiae, S. bayanus*, and *S. paradoxus* can be distinguished by using *Hae*III and *Hpa*II endonucleases [15, 16].

Precise identification of the new strains was performed by amplification of the 5.8S-ITS fragment in the two isolates and in three reference strains, which served as species standards. The size of the PCR product was the same for both of the new isolates and was 850 bp (Fig. 2), which is typical for the genus *Saccharomyces*.

The PCR products were analyzed by restriction with *Hae*III and *Hpa*II endonucleases (Fig. 3) using reference strains *S. cerevisiae* CBS 1171, *S. paradoxus* CBS 432, and *S. bayanus* CBS 380.

Restriction endonuclease analysis of the *S. cerevisiae* strains revealed four *Hae*III-generated fragments with sizes of 320, 230, 170, and 130 bp and two *Hpa*II-generated fragments with sizes of approximately 730 and 120 bp. *S. paradoxus* strains were earlier shown to lack *Hpa*II-restriction sites and to have *Hae*II-restriction profiles identical to those of *S. cerevisiae* strains [15, 16].

Thus, based on their restriction profiles, both of our isolates from western Siberia should be assigned to *S. paradoxus.*

It should be noted that this paper is the first to be presented on the isolation of *S. paradoxus* in western Siberia. Probably, the isolation of a strain belonging to this species was successful due to the fact that the oak exudate was sampled at the end of the summer and not in spring, the period of most intense of exudation, when isolation of saccharomycetes is usually conducted. The isolation *S. paradoxus* can testify to the continuity of the natural habitat of this species. Repeated discovery of new strains of *S. paradoxus* in this region should allow the characterization of this as yet unstudied population and expand our knowledge on the geographical distribution of yeasts of the genus *Saccharomyces* in nature.

ACKNOWLEDGMENTS

The author is grateful to G.I. Naumov and E.S. Naumova their help in the analyses and their attention to this work.

Molecular identification of the strains was carried out at the Laboratory of Molecular Genetics of the State Research Institute of Genetics and Selection of Industrial Microorganisms with financial support from the federal targeted program "Integration of Science and Higher Education in Russia in 2002–2006" and from the Russian Foundation for Basic Research, project no. 04-04-48713.

REFERENCES

1. Kurtzman, C.P., Phylogenetic Circumscription of *Saccharomyces, Kluyveromyces* and Other Members of the *Saccharomycetaceae*, and the Proposal of the New Genera *Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma* and *Zygotorulaspora, FEMS Yeast Research*, 2003, vol. 4, no. 3, pp. 233–245.

2. Naumov, G.I. and Nikonenko, T.A., New isolates of *Saccharomyces paradoxus* from Oak Exudates, *Dokl. Akad. Nauk SSSR, Ser. Biol.,* 1988, no. 7, pp. 84–87.
3. Naumov, G., Naumova, E., and Sniegowski, P., Differentiation of European and East Asian Populations of *Saccharomyces paradoxus* by Allozyme Analysis, *Int. J. Bacteriol.*, 1997, vol. 47, pp. 341–344.
4. Naumov, G.I., Naumova, E.S., and Molina, F.I., Genetic Variations among European Strains of *Saccharomyces paradoxus:* Results from DNA Fingerprinting, *Syst. Appl. Microbiol.*, 2000, vol. 23, pp. 86–92.
5. Naumov, G.I., Naumova, E.S., Azbukina, Z.M., Korhola, M., and Gaillardin, C., Genetic and Karyotypic Identification of *Saccharomyces* Yeasts from Far East Asia, *Cryptogamie Mycol.*, 1993, vol. 14, pp. 85–93.
6. Naumov, G.I., Divergent Population of the Yeast *Saccharomyces paradoxus* on the Hawaiian Islands: Species *In Statu Nascendi, Dokl. Akad. Nauk, Ser. Biol.,* 1999, vol. 364, no. 2, pp. 281–283.
7. Naumov, G.I., Naumova, E.S., and Sniegowski, P.D., *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* Are Associated with Exudates of North American Oaks, *Can. J. Microbiol.*, 1998, vol. 44, pp. 1045–1050.
8. Yurkov, A.M., Maksimova, I.A., and Chernov, I.Yu., Comparative Analysis of the Structure of Yeast Communities in Birch Forests of the European Russia and Western Siberia, *Mikol. Fitopatol.*, 2004, vol. 38, no. 6, pp. 71–79.
9. Naumov, G.I. and Naumova, E.S., Discovery of a Wild Population of Yeasts of the Biological Species *Saccharomyces cerevisiae* in Siberia, *Mikrobiologiya*, 1991, vol. 60, no. 3, pp. 537–540.
10. Barnett, J.A., Payne, R.W., and Yarrow, D., *Yeasts: Characteristics and Identification. 2nd Ed.*, Cambridge: Cambridge University Press, 1990.
11. *The Yeasts, a Taxonomic Study. Fourth Revised and Enlarged Edition*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier Science B.V.,1998.
12. Naumova, E.S., Korshunova, I.V., Jespersen, L., and Naumov, G.I., Molecular Genetic Identification of *Saccharomyces* Sensu Stricto Strains from African Sorghum Beer, *FEMS Yeast Res.*, 2003, vol. 3, pp. 177–184.
13. Naumov, G., Naumova, E., Sancho, E., and Korhola, M., Taxogenetics of the *Saccharomyces* Sensu Stricto from Western and South Africa, *Cryptogamie Mycol.*, 1993, vol. 14, no. 4, pp. 263–270.
14. Naumov, G., Naumova, E., Hagler, A., Mendonca-Hagler, L., and Louis, E., A New Genetically Isolated Population of the *Saccharomyces* Sensu Stricto Complex from Brazil, *Antonie van Leeuwenhoek*, 1995, vol. 67, pp. 351–355.
15. Naumov, G.I., James, S.A., Naumova, E.S., Louis, E.J., and Roberts, I.N., Three New Species in the *Saccharomyces* Sensu Stricto Complex: *Saccharomyces cariocanus, Saccharomyces kudriavzevii* and *Saccharomyces mikatae, Int. J. Syst. Evol. Microbiol*, 2000, vol. 50, pp. 1931–1942.
16. Naumov, G.I., Gazdiev, D.O., and Naumova, E.S., Discovery of the Biological Species *Saccharomyces bayanus* in Far East Asia, *Mikrobiologiya*, 2003, vol. 72, no. 6, pp. 834–839.