Temperature-Dependent Dimorphism of the Non-Conventional Yeast *Arxula adeninivorans*

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Aim

Analysis of the temperature-dependent dimorphism of *A. adeninivorans* LS3.

Introduction

A. adeninivorans is a nonpathogenic, ascomycetous, haploid, anamorphic, arthroconidial yeast characterized by special features such as temperature-dependent dimorphism, thermo- and salt resistance (Kunze and Kunze 1996, Wartmann and Kunze 2000). *Arxula* cells can be cultivated at temperatures up to 48 °C in media containing as much as 20 % NaCl. This yeast is also able to utilize a single energy and carbon source from a range of compounds, including adenine, uric acid, starch and others.

The temperature-dependent dimorphism of *A. adeninivorans* is of particular interest. Wartmann et al. (1995) discovered that culturing this yeast at elevated temperatures (higher than 42 °C) induces a gene expression pattern that results in a morphological transition from budding to mycelial form. Wild-type strain LS3 forms budding cells up to a temperature of 41 °C, pseudomycelia at 42 °C and mycelia at 43 °C and higher temperatures. This dimorphism is reversible and the budding phenotype is restored when the culturing temperature is lowered to 42 °C or below. Dimorphism and

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special molecular biological characteristics make *A. adeninivorans* an attractive organism for study (Gienow et al. 1990). Research is facilitated by the haploid state that provides an easy approach to selection of mutants (Samsonova et al. 1996).

For a more detailed analysis of dimorphism, mutants with altered dimorphism characteristics have been selected enabling discrimination between dimorphism and temperature effects (Wartmann et al. 2000). These mutants were identified by colony form. Budding cells form smooth colonies, pseudomycelia rough colonies, and mycelia exclusively very rough colonies (Fig. 1).

Materials

Strains

- *A. adeninivorans* LS3 – wild-type (Kunze and Kunze 1994)
- *A. adeninivorans* 135 – mutant with altered dimorphism characteristics (Wartmann et al. 2000)

Media

- YEPD medium: 0.5 % peptone, 0.5 % yeast extract, 2 % glucose
- YEPD agar: YEPD medium with 2 % agar
- One litre of YMM medium (Tanaka et al. 1967) contains 3.7 g $NaNO_3$, 8.4 g KH_2PO_4, 1.0 g $MgSO_4$, 2.0 mg $Ca(NO_3)_2$ 4 H_2O, 0.2 mg $FeCl_3$ 6 H_2O, 1 %

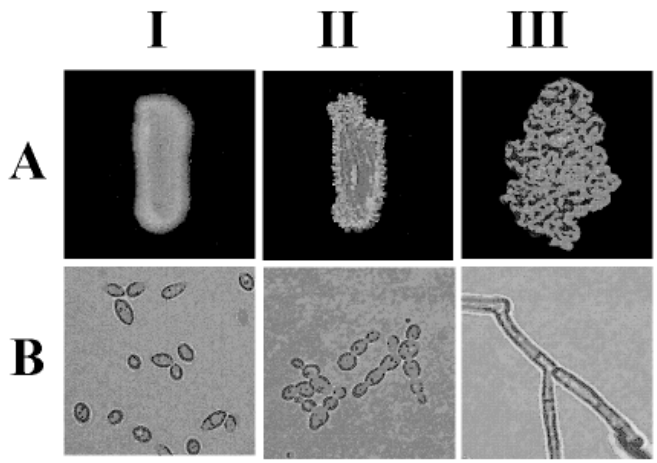

Fig. 1. **A** Colony form and **B** Cell morphology of *A. adeninivorans* LS3 grown at 30 °C (*I*), 42 °C (*II*), and 45 °C (*III*). Cells were cultured in YEPD medium for 18 h

glucose and 1 mL of a component II with 50 mg H_3BO_4, 10 mg $CuSO_4 \cdot 4\ H_2O$, 10 mg KJ, 40 mg $MnSO_4 \cdot 4\ H_2O$, 40 mg $ZnSO_4 \cdot 7\ H_2O$, 20 mg Na_2MoO_4, 10 mg $CoCl_2$ per 100 mL Aqua dest. Before using the YMM 0.05 vol vitamin mix solution, add 40 mg Ca-D-pantothenate, 40 mg thiamine dichloride, 10 mg nicotinic acid, 40 mg pyridoxine, 0.4 mg biotin, and 400 mg inositol per 100 mL.

- YMM agar: YMM medium with 2 % agar

Reagents

- 0.9 % NaCl
- *N*-Methyl-*N′*-nitro-*N*-nitrosoguanidine solution: 0.4 mg mL^{-1} *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine in 0.9 % NaCl

Experimental Procedure

Day 1

Starting an overnight culture of 200 mL of the *A. adeninivorans* strains LS3 in YEPD medium at 30 °C.

Day 2

The yeast culture should contain approximately 10^8 cells mL^{-1}. The *A. adeninivorans* cells are harvested, washed with water and resuspended in 0.9 % NaCl. Then cell clusters are carefully disrupted by sonification. The resulting suspension is adjusted to 10^8 cells mL^{-1} and then treated with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine for 2 h at 20 °C. Three washing steps with 0.9 % NaCl follow, before cells are spread onto YMM agar plates and cultured at 30 °C for 3 days.

Day 5

Colonies that exhibit an altered surface (rough and very rough) as compared with the strain LS3 (smooth colonies) are putative mutants with altered dimorphism charcateristics. They are selected and characterized. For this purpose the mutant cells are cultured in 10 mL YMM at 30 °C, 42 °C and 45 °C for 20 h.

Day 6

All selected mutants are inspected by microscopy to confirm the altered dimorphism characteristics. Cells originating from colonies with rough surfaces should grow as pseudomycelia and those originating from colonies with very rough surfaces as mycelia at 30 °C. Shifting cultivation conditions to higher temperatures should induce formation of mycelia in all strains including wild-type strain LS3.

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