The Isolation of Obligate Osmophilic Mutants of the Yeast *Saccharomyces rouxii*

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INTRODUCTION

Osmophilic yeast was first defined by von Richter (1912) as a yeast capable of growing well in environments of high osmotic pressure. Since then, many yeasts able to grow in high concentrations of sugar or salt have been isolated. None has shown an obligate requirement for high sugar or salt concentrations for growth, except at elevated temperatures for certain strains (Onishi, 1963*a*). The temperature-conditional obligate osmophiles described by Lochhead & Heron (1929), Spencer & Salans (1956) and Onishi (1963*b*) were strains of *Saccharomyces rouxii*, *S. rouxii* and *Torulopsis halonitratophila*, respectively. In the present study several obligate osmophilic mutants of *S. rouxii*, an osmotolerant yeast, have been isolated.

METHODS

A culture of *Saccharomyces rouxii* Boutroux (sa 24), isolated by Dr M. P. Scarr (Scarr & Rose, 1966), was grown to mid-exponential phase (approx. 1 mg/ml), in defined medium (D medium) (Davies, 1964) supplemented with 40 % (w/v) sucrose. Freshly prepared *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Sigma, 2 mg/ml) was added to a final concentration of 200 µg/ml and the culture incubated at 30 °C for 30 min. Next, 0.2 vol. of cold 0.6 M-KCl was added and the yeast separated at 37000 *g* for 5 min at 4 °C. The cells were then washed in the centrifuge six times with a mixture of cold growth medium and 0.6 M-KCl (5:1, v/v). The organisms were counted in a haemocytometer and suspended in the growth medium at a density of 10⁵ cells/ml (approx. 10³ viable cells/ml). Portions (0.2 ml) were plated on yeast extract (Difco) agar supplemented with 50 % (w/v) glucose, with 10 % glucose plus 40 % sucrose, or with 2 % glucose plus 20 % KCl, and incubated at 30 °C.

After 7 days, the primary plates were replica-plated on the corresponding agar plates and also on 2 % (w/v) glucose-0.2 % yeast extract (Difco) agar plates. The plates were incubated at 30 °C for 7 days and each pair compared. The organisms which grew at high, but not at low, solute concentrations were resuspended in a small volume of D medium and streaked on both high and low solute-wort agar (Oxoid) to test for osmophilic properties. Strains showing a requirement for high solute concentration in a repetition of the above tests were maintained on the high solute-wort agar medium. The obligate osmophilic character of the mutants was also shown in liquid medium.

In the other tests the yeasts were first grown on D-medium agar supplemented with 2 % (w/v) glucose plus 40 % sucrose, and then streaked on agar medium containing either a 0.1 or a 1.2 M solution of one of the following as sole carbon source: glucose, galactose, mannose, sucrose, maltose, lactose, glycerol, mannitol, sorbitol or sodium lactate.

Table 1. Growth of *S. rouxii* and its obligate osmophilic mutants with various compounds as sole carbon source, in the presence of various concentrations of solutes

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The disaccharides were sterilized by filtering a 50 % (w/v) solution at 60 °C through a Seitz filter, and then mixed at 60 °C with 0.25 vol. of sterile D-medium agar of 4 times the normal concentration.

Adaptation to growth in low solute concentrations was studied by culturing the yeast successively in 40, 20, 10 and 0 % (w/v) sucrose dissolved in the 2 % glucose-D-medium agar.

RESULTS AND DISCUSSION

Three series of mutants were selected according to their ability to grow in yeast extract medium containing 10 % (w/v) glucose plus 40 % (w/v) sucrose (series A), 50 % (w/v) glucose (series B), or 25 % (w/v) glucose plus 20 % (w/v) KCl (series C). None grew in 2 % (w/v) glucose (Table 1). In further contrast to the parent, *S. rouxii*, the mutant strains failed to grow when a 0.1 M solution of glucose, galactose, mannose, maltose, lactose, mannitol or glycerol was used as sole carbon source (Table 1). Seven of the mutants were adapted to growth in 2 % glucose during subculture in decreasing concentrations of sucrose. Of the remainder, twenty-six failed to grow in concentrations of sucrose of up to 10 %, and ten strains in concentrations of up to 20 %. All the C series of mutants grew best with 10 % KCl. Five of the A and B series were unable to grow in 2 % glucose plus 5 to 20 % KCl, while the others showed optimal growth in various KCl concentrations. On yeast extract-agar, three of the mutants grew on 0.6 M-glycerol, seven on 1.2 M-, 20 on 2.4 M- and a further six grew weakly on 2.4 M-glycerol. At least eight of the mutants did not grow at any glycerol concentration in the range 0.1 to 2.4 M. Nine strains grew in liquid defined medium containing 0.6 M-glycerol. Growth of the parent *S. rouxii* occurred over the whole range of glycerol concentrations used.

None of the mutants grew on sucrose or lactose, but all except three grew weakly on 40 % (w/v) maltose. With one exception all failed to grow on 1.2 M-sodium lactate and only three grew on 0.6 M-mannitol (two of them poorly). The parent *S. rouxii* grew poorly on maltose, sucrose or lactose. It grew on sodium lactate and on mannitol. The original strain of *S. rouxii* and all but six of the mutants grew on 40 % galactose. Some of the mutants reverted to growth in 2 % glucose during repeated subculture on yeast extract agar containing 2 % glucose plus 40 % sucrose.

The length (5 to 15 μ m) and ovoid shape of the parent *S. rouxii* organism was not changed by growth at high or low concentrations of sucrose. When grown in the presence of 40 % sucrose, many of the mutants were similar in size and shape to the parent organisms. Some were spherical and larger than the parent (Table 1) and some (e.g. strains B1/4, C1/5 and A2/9) extruded membranous material, detected under phase-contrast microscopy, when the yeast was subjected to a sudden decrease in osmotic pressure (Table 1).

Most of the obligate osmophilic mutants of *S. rouxii* isolated in the present study showed some form of weakness in the cell envelope. All the mutants were unable to grow in glycerol concentrations below 0.6 M. Bearing in mind that the extent to which yeast membranes are permeable to glycerol is uncertain, it seems possible that the lesion (i) affects osmotic regulation, (ii) affects respiratory deficiency, or (iii) reflects a requirement for high solute or low water activities inside the cells.

Nitrosoguanidines have been found to cause multiple mutations in bacteria (Adelberg, Mandel & Chein, 1965). The ability of some of the *S. rouxii* mutants to revert and of some to adapt to growth in 2 % glucose are indications that these are not multiple mutants. No revertant has been isolated from strain B1/4 and hence this strain may result from multiple mutation. Genetic studies with *S. rouxii* are difficult since it is heterothallic, normally in

the haploid state, does not form diploids readily, and hence is unable to form ascospores (Wickerham & Burton, 1960). However, English (1954) was able to isolate spores from one strain of *S. rouxii*.

Some of the chemical changes associated with the osmotic fragility and unusual morphology of mutant B1/4 (Table 1) are the subject of an accompanying paper (Koh, 1975).

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