An Obligate Osmophilic Yeast from Honey

M. T. MUNITIS, E. CABRERA, AND A. RODRIGUEZ-NAVARRO*

Catedra de Microbiologia, Escuela Tecnica Superior de Ingenieros Agronomicos, Madrid 3, Spain

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An obligate osmophilic yeast that requires high sugar concentrations (10 to 20% glucose) for growth was identified as Saccharomyces bisporus var. melis. Optimum growth for this strain was at 60% glucose. Several non-assimilable compounds permitted growth at glucose concentrations below the minimum requirement and stimulated growth at glucose concentrations above the minimum. No correlation existed between growth stimulation and spheroplast stabilization capacities of the compounds examined.

To determine generation times, 10^2 cells from a culture in 30% glucose were used to inoculate 200-ml Erlenmeyer flasks with 50 ml of the appropriate medium. The flasks were incubated on an orbital shaker, and growth was followed turbidimetrically at 550 nm, up to a cell density of 0.5 ml of cell dry weight per ml. From the above flask an inoculum was transferred into fresh medium, and the generation time was again determined.

Preparation of spheroplasts. Spheroplasts were prepared according to Wiley (17), using snail juice enzymes (Helicase, Industrie Biologique Francaise, S.A.). Stabilizers used and their concentrations are given in Results.

Manometry. Anaerobic fermentation was measured manometrically as described by Umbreit et al. (12). Cells were grown on basal medium plus 30% glucose and were collected at the middle of the logarithmic phase.

RESULTS

Identification characteristics. After 3 days on GO agar, cells were ovoid or ellipsoidal (3 to 5 by 6 to 8 μm), occurring singly or in pairs. In 30% glucose liquid medium, cells were similar in shape to those on GO agar, and a sediment was formed.

Sporulation after conjugation was observed in the dry part of a slant of V-8 juice agar. Two to four round ascospores were formed per ascus.

Glucose was fermented, but maltose, sucrose, and galactose were not. Sucrose, galactose, and L-arabinose were assimilated. Weak growth was observed on erythritol, sorbitol, and glycerol, and no growth was observed on maltose, xyllose, and sorbose. Assimilation of potassium nitrate was negative.

Concentrations of sugars and sugar alcohols required for growth. Strain 0.11-1 did not grow at glucose concentrations lower than 10%, and at concentrations from 10 to 20% it grew at unmeasurable rates. Increasing rates were obtained when the glucose concentration was in-
increased further (optimum at about 60%). At equal levels, growth on fructose was slower than that on glucose at concentrations of up to 40%, and it was faster on fructose at higher concentrations (50 to 70%) (Table 1).

Glycerol stimulated the growth of strain 0.11-1 (Table 1). At 1% glucose, glycerol promoted growth if added above the 15% level, but growth rates were lower than on glucose alone at similar water activities (water activities were taken from reference 6), i.e., 30% glycerol plus 1% glucose versus 50% glucose (doubling time, 8.6 h versus 5.4 h). The maximal growth rate on 1% glucose plus glycerol was also lower than the maximal on glucose alone (doubling time, 7.6 h versus 5.2 h), but the optimum water activities were similar in both cases (40% glycerol and 60% glucose). At 10% glucose, stimulation by glycerol was the same as stimulation by glycerol at levels yielding similar water activities; i.e., growth rate on 10% glucose plus 30% glycerol was not different from that on 60% glucose.

Sorbose, sorbitol, and xylose promoted a slow growth at 1% glucose and a faster one at 10% glucose, but none of these compounds was as effective as glycerol (Table 1).

An increase in temperature from 18 to 34°C slightly increased the minimum concentration of glucose requirement. No growth was observed in any case under 18°C.

Effect of KCl on growth. KCl did not promote growth of strain 0.11-1 at a glucose concentration under 3%. At a 3 to 5% glucose concentration, 1 M KCl promoted growth but not at measurable rates. At higher concentrations of glucose, KCl stimulated growth if used at concentrations under a certain level, a level dependent on the concentration of glucose (Table 1). Growth rates similar to those on optimum glucose (60%) were obtained with 0.5 and 1 M KCl at 30 and 40% glucose.

The effects of KCl not only were dependent on glucose concentrations but also were partially dependent on other solutes present in culture media (Table 1); 0.5 M KCl plus 20% sorbose and 1% glucose supported growth, whereas any combination of two of them at the stated concentrations did not.

Effects of other compounds. Polyethylene glycol (PEG) 200 (Merck) proved to be a stimulant for the growth of strain 0.11-1 at glucose concentrations above the minimum, provided that the PEG was used at under a 40% concentration (25% optimal). A measurable rate at 1% glucose was obtained with the addition of 30% PEG (Table 1). Some toxicity of PEG was observed even under good growth conditions (10% glucose plus 25% PEG), as judged by the atypical shape and abnormal budding behavior of the cells.

Ethylene glycol (1 M), which has been reported to repair the mutant phenotype in osmotic remedial mutants (2), inhibited growth of strain 0.11-1 at all glucose concentrations.
Sodium taurocholate (1 to 4%), Tween 80 (1.0 ml/liter), or ethanol (5 to 14%), which have been found to be stimulatory for an osmophilic yeast that grew slowly in 1% glucose (3), did not promote growth of our strain.

**Spheroplast stabilization.** Data in Table 2 show the effectiveness of sorbitol and KCl at several concentrations on maintaining the integrity of spheroplasts of strain 0.11-1 grown in 30% glucose. Glycerol and glucose were not suitable stabilizers for spheroplasts (data not shown).

When cells grown on 30% glucose were adapted to low sugar concentrations by keeping them in 10% glucose medium for 2 h and then were transferred to 2% glucose medium, the required concentrations of stabilizers for spheroplasts were still the same. A similar KCl requirement for stabilization of spheroplasts has been reported for a different osmophilic yeast (16).

**Fermentation of glucose.** Strain 0.11-1 grown on 30% glucose fermented anaerobically even in the absence of added substrate, and increasing concentrations of glucose up to 40% slightly increased CO₂ production. When yeast was starved for 5 h in potassium phosphate buffer (20 mM, pH 7) plus a stabilizer, different responses were observed. (i) Sorbose (30%), glycerol (17%), xylose (25%), and sorbitol (30%) delayed fermentation of 30% glucose for 5 to 15 min, and then fermentation started and increased, eventually reaching a constant value in 2 to 3 h. (ii) KCl (0.25 M) delayed fermentation for 4 to 6 h, taking several hours more to reach a constant value. (iii) PEG 200 (25%) allowed fermentation without lag, but at much slower rates than with the above compounds.

**DISCUSSION**

Most of the osmophilic yeasts are included in the species Saccharomyces rouxii, S. bailii var. osmophilus, and S. bisporus var. mellis, the last two species being distinguished on the basis of their cellular size (14). Results of fermentation and assimilation tests of strain 0.11-1 are coincident with those reported for S. bailii and S. bisporus, although the comparison of our results, obtained at 30% concentrations of carbon compounds, with those obtained for these species at 0.5 and 2% may be questioned. It must be noted that our strain did not grow even at a 10% concentration of carbon compounds, which is a recommended concentration for the classification tests of osmophilic yeasts (10). Cell size may be of little differential value, however, because it could be affected by the high concentration of glucose in the medium (9). Based on the similarity of the morphologi-

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<th>Stabilizer</th>
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<tr>
<td>Sorbitol</td>
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<td>Lysis</td>
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<td>3.00</td>
<td>Spheroplasts, whole cells</td>
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* Cells were treated with Helicase for 30 min in 20 mM phosphate buffer, pH 6.0 (17).

The failure of KCl to promote growth at glucose concentrations under 3% may be due to a low stimulation capacity that cannot be explained in terms of water activity; only specific effects could account for the fact that KCl promoted growth on 20% sorbose plus 1% glucose and not on 1% glucose.
There is a lack of correlation between growth stimulation by a certain compound and its ability to stabilize spheroplasts. Glycerol was a better stimulator but a worse stabilizer than KCl. This seems to indicate that growth stimulation does not consist of an osmotic stabilization of the cell. Permeable solutes have also been observed to repair the mutant phenotype in osmotic remedial mutants (2, 4).

Anand and Brown (1), after observing the growth rate patterns of osmophilic yeasts in solutions of PEG, did not find evidence of a general requirement for decreased water activity of osmophilic yeasts and considered the suffix "philic" as inaccurate, proposing instead the term "sugar tolerant" for these yeasts.

The designation of "philic" for strain 0.11-1 seems accurate, but what is less clear is whether the strain is "osmophilic" or merely "saccharophilic." As we have mentioned before, glucose, and probably other assimilable sugars, may be an important factor in determining the peculiar physiology of the strain. However, non-assimilable sugars and other compounds, sugar related or not, were stimulatory to the strain. Solutions of sorbose, KCl, and PEG share few properties, except that they decrease the water activity of the medium, but growth of strain 0.11-1 was still greatly stimulated with these compounds, provided that 1 to 5% glucose was present.

The above results seem to warrant the use of the term "osmophilic" to describe the behavior of our strain, in spite of the solute specific effects. We therefore conclude that strain 0.11-1 is an obligate osmophilic yeast.

LITERATURE CITED


