Interspecific Protoplast Fusion of *Saccharomyces cerevisiae* and *Saccharomyces mellis*

R. Legmann and P. Margalith

Department of Food Engineering and Biotechnology, Technion, Israel Institute of Technology, Haifa, 32000, Israel

**SUMMARY**

Protoplasts of auxotrophic mutants of the highly fermentative *Saccharomyces cerevisiae* and the osmotolerant *S. mellis* were fused. The frequency of appearance of prototrophic hybrids was 0.75-1.6 per 10⁶ protoplasts. Biochemical analysis of the stable hybrids revealed the characteristics of both parents in about 50% of the recombinants. Fermentation at high glucose concentrations by some of the hybrids was considerably improved.

**INTRODUCTION**

Although raw materials are the principal expenditure in the industrial alcoholic fermentation, energy savings must also be considered. Increasing the ethanol content of fermentation washes would reduce considerably distillation costs (Stewart, 1981, Holcberg & Margalith, 1981). In order to increase the ethanol concentration, higher sugar concentrations must be employed. These, however, would reduce the fermentation rate. In order to overcome this obstacle, it was suggested to form a new hybrid of yeast which would contain the characteristics of an osmo-tolerant organism with that of a highly fermentative yeast.

**MATERIALS AND METHODS**

Organisms: *S. cerevisiae*, haploid strains, type a (H-227) lysineless, and type α (H-70) threonineless, were obtained from the Department of Genetics of the Hebrew University, Jerusalem.

*S. mellis*, strain T453, was originally isolated from concentrated grape juice (650°Bx). Auxotrophic mutants, requiring methionine and arginine, were obtained by UV irradiation. All strains were maintained on yeast extract-peptone-glucose media.

Protoplast formation: The preparation of protoplasts of *S. cerevisiae* was performed according to Maraz and Ferenczy (1979). Protoplasts of *S. mellis* were prepared according to Arnold and Garrison (1979). In both cases the cell wall digestion was performed with Zymolase 5000, 0.3 mg/ml (Kirin Brewery Co., Japan).

Protoplast fusion and cell regeneration: A 1:1 mixture of both protoplasts were suspended in 1 M sorbitol and treated with polyethylene-glycol (PEG; M.W. 6000) and CaCl₂ according to Fournier et al (1977). After incubation for 20 min. with gentle shaking at 28°C, protoplasts were serially diluted in sorbitol (1 M) in phosphate buffer (0.1 M), pH=6.8. Suitable dilutions were introduced into molten minimal (OMM) and complete agar (OYEG) prepared according to Maraz and Ferenczy (1979) and overlaid in petri dishes containing pre-solidified media of similar composition. Control plates for the determination of back mutations were also included. A mixture of both types of protoplasts without PEG served as control for possible cross feeding. Plates were incubated at 30°C. Colony formation was followed during 7 days. Fusion frequency was calculated according to Chepurnaya et al (1980).

**RESULTS AND DISCUSSION**

Protoplast yields were 95 to 99% with both yeasts. The fusion frequency between the
Fig. 1. Raffinose assimilation by parent and hybrid strains. 
- - S. cerevisiae lys<sup>-</sup>; - - S. mellis met<sup>-</sup>; - - Hybrid, No. 4; 
- - - Hybrid, No. 12; - - - Hybrid, No. 69. Growth was followed 
in complete medium (pH = 7.0) containing raffinose (2%) as sole 
carbon source. Incubation at 30°C with vigorous shaking.

Fig. 2. Growth of parents and hybrids on 49% (w/w) glucose agar. 
Top row: left = S. cerevisiae, lys<sup>-</sup>; right = S. mellis, met<sup>-</sup>; Bottom 
row: left = hybrid No. 69, right = hybrid No. 12. 10 days incubation 
at 30°C.

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**TABLE 1: Interspecific Fusion of Saccharomyces cerevisiae and S. mellis**

<table>
<thead>
<tr>
<th>Parents</th>
<th>Protoplast Reversion frequency</th>
<th>Fusion frequency</th>
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<tbody>
<tr>
<td>S. cerevisiae</td>
<td>S. mellis</td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>a lys&lt;sup&gt;-&lt;/sup&gt;</td>
<td>x met&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1.7 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>a thr&lt;sup&gt;-&lt;/sup&gt;</td>
<td>x arg&lt;sup&gt;-&lt;/sup&gt;</td>
<td>2.8 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
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**TABLE 2: DNA Content of Parent and Hybrid Strains**

<table>
<thead>
<tr>
<th>Parent</th>
<th>DNA concentration (µg/cell)</th>
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<tbody>
<tr>
<td>S. cerevisiae (H-227)lys&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1.27 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. mellis met&lt;sup&gt;-&lt;/sup&gt;</td>
<td>2.04 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hybrids: No. 69</td>
<td>3.39 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. 12</td>
<td>3.36 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
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Results are the average of 4 determinations.

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different organisms may be seen in Table 1. Raffinose assimilation is described in Fig.1. Growth on high glucose agar can be appreciated from Fig. 2. From these figures it can be seen that in the hybrids examined the osmo-tolerant property was indeed fused to the raffinose fermenting character. From a total of 34 recombinant prototrophs isolated from OMM plates, 9 proved to be stable (for over 10 months) hybrids capable of growth at high sugar concentrations and raffinose utilization like their respective parents. Fermentation experiments are described in Fig. 3. As can be seen some of the hybrids (nos. 12 and 69) were able to ferment at high sugar concentrations better than their corresponding parents.