A NOVEL PROCEDURE FOR THE RECOVERY OF HYBRID PRODUCTS FROM PROTOPLAST FUSION

R. Vidoli¹, H. Yamazaki¹, A. Nasim and I.A. Veliky*

Carleton University¹ and National Research Council Ottawa, Canada K1A OR6

SUMMARY: A protoplast mixture of two $\alpha$-mating type strains of Saccharomyces cerevisiae was encapsulated in a beaded form of calcium alginate gel. Part of the same protoplast mixture was embedded in a molten (50-55°C) agar overlay, a routine procedure for the recovery of regenerated colonies and hybrid products. The number of hybrid products isolated from the encapsulated protoplast mixtures was greater than that recovered from the agar overlay. The increase ranged from 40 to 1000 fold.

INTRODUCTION: Protoplast fusion is a useful tool for asexual genetic manipulation of various microorganisms and as such it is a potential technique for strain improvement in microbes (Peberdy, 1980; Ferenczy, 1981). Intraspecific fusions produce stable hybrids with frequencies ranging from $10^{-3}$ to $10^{-5}$ (Christensen, 1979; Fournier, 1977; Sarachek, 1981; Ferenczy, 1976; Hockney & Freeman, 1980). Any procedure that can lead to an increase in the number of recovered hybrid products can be useful in wider applications. This paper reports a novel approach for the recovery of hybrid products using encapsulation of protoplast mixtures in metal-ion gelled polysaccharide matrices. Hybrid yields by using the encapsulation technique are much higher than those obtained by the routine agar overlay method.

MATERIALS AND METHODS: Yeast strains: Saccharomyces cerevisiae XY207-5C $\alpha$-ade-leu-his and Saccharomyces cerevisiae CS40-5B $\alpha$-ade-arg-try were used in the fusion experiments (strains were kindly provided by Dr. A. James).

Protoplast fusion: Cultures were grown overnight in 20 ml YEPD medium (1% yeast extract, 2% peptone, 2% dextrose) at 30°C on a shaker (150 rpm), centrifuged and resuspended in YEPD medium to give 10^6 cells/ml (total volume 10 ml).
The resulting suspension was grown at 39°C on a shaker for 3 hours. The logarithmic phase cells were centrifuged, washed twice with sterile water, once with 1.2 M solution of sorbitol, and centrifuged. The washed cells were resuspended in 10 ml 1.2 M solution of sorbitol. The cell suspension was treated with zymolyase-60,000 (40 μg/ml) for 10 minutes at 30°C with shaking (75 rpm) to obtain protoplasts. The protoplasts were gently washed 3 times with 1.2 M solution of sorbitol centrifuged each time for 5 minutes at 2000 rpm, and finally resuspended in 0.25 ml 1.2 M sorbitol in 10 mM calcium chloride, pH 8.

Two mixtures, both containing 0.1 ml protoplast suspension of each strain, were left at 21°C for 15 minutes. After 15 minutes, 2 ml of 20% polyethylene glycol (PEG), MW 4000, and 10 mM calcium chloride in 10 mM TRIS-HCl buffer solution pH 8 was slowly added to each of the mixtures, mixed thoroughly, left at 21°C for 20 minutes, and centrifuged 5 min at 2000 rpm.

RESULTS AND DISCUSSION: Table I shows the results of a typical fusion experiment comparing the conventional agar overlay procedure to the novel procedure of alginate encapsulation. Regenerated colonies and hybrid products can be recovered using the alginate encapsulation procedure after a 4 day incubation period.