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PROTOPLAST FUSION

SUKANYA PONGTHONG : COMPARATIVE STUDY OF ELECTROFUSION AND CHEMICAL
FUSION OF YEAST PROTOPLASTS. THESIS ADVISOR : ASSIST. PROF. MANA
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Comparative studies of protoplast fusion between Endomycopsis fibuligera ATCC 9947, an amylases-producing strain, and Candida oleophila NNU62, a citric acid-producing strain, by electrofusion and by treatment with polyethylene glycol (PEG) were performed. Suitable conditions for protoplast formation from both strains were by incubating at 35°C for 30 min. in zymolyase at 0.1 mg/ml for E. fibuligera and at 0.4 mg/ml for C. oleophila.

Protoplast fusion by both methods yielded strains capable to produce higher level of either amylases or citric acid than those of the parental strains. However by using electrofusion, strains with ability to produce both amylases and citric acid were obtained but they were unstable after subculturing.

Electrofusion conditions yielding strains capable to produce higher amylases than that of E. fibuligera were with direct current of 10 square pulse with 10 s pulse width and field strength at 5 kV/cm in 0.7 M sorbitol containing 0.5 mM Ca^{2+} and 0.7 mM Mg^{2+} . Under these conditions, fusion frequency of 0.07 was obtained. Conditions for electrofusion giving strains producing higher citric acid than that of C. oleophila were similar to the above except for the fusion solution contained 0.7 mM Ca^{2+} and 0.1 mM Mg^{2+} . Fusion frequency under these conditions was 0.2.

Suitable conditions for protoplast fusion by treating with PEG were at 20°C for 20 min, under these conditions only strains capable to produce citric acid but higher than that of C. oleophila with fusion frequency of 0.20 were obtained.

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