DEVELOPMENT OF A TRANSFORMATION SYSTEM OF Mucor rouxii ATCC 24095 BY ELECTROPORATION OF YEAST-LIKE CELL

Mucor rouxii is a dimorphic fungus that is capable of production of an essential fatty acid, gamma linolenic acid (6,9,12-cis-octadecatrienoic acid, GLA). We have developed the transformation system of M. rouxii for facilitating molecular genetic studies as well as for strain improvement. Genetic transformation of M. rouxii was employed using two plasmids namely pHL.DD and pD4. The pHL.DD is an artificial chromosomal plasmid carrying the gpdA promoter of Aspergillus nidulan and telomeric sequence. The pD4 is an integrative plasmid consisting of H4 histone promoter and ribosomal DNA region of Mortierella alpina. These plasmids were transformed either by electroporation or polyethyleneglycol-mediated method. The transformants were selected on complete medium containing hygromycin B at a concentration of 200 µg ml⁻¹. Polymerase chain reaction was used for the analysis of existence of the transformed DNA. There is no significant difference in the efficiency of transformation with the two methods. The transformation frequency was between 5-10 transformants/10⁷ cells/5 µg DNA. The examination of mitotic stability of transformants showed that the hygromycin resistant phenotype of the pD4 transformant was stable for three serial subculturing on non-selective medium while pHL.DD transformant exhibited a high degree of instability.