

## MUTATION AND PROTOPLAST FUSION OF *CEPHAROSPORIUM EICHHORNIAE*

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Strains of desired characters could be obtained by genetic engineering. Mutation and protoplast fusion are other techniques used in strain improvement. *Cepharosporium eichhorniae* an industrially important fungus was selected for the mutation and protoplast fusion studies. For protoplast fusion, two double auxotrophic mutants of *C. eichhorniae* were required. Hence *C. eichhorniae* 822, a single auxotrophic mutant for the amino acid methionine was mutated using nitroso-guanidine. The survival and mutation frequencies were 9.9% and 16.4% respectively. Out of the 81 mutants obtained, a double auxotrophic mutant for the amino acids methionine and proline was selected for protoplast fusion with *C. eichhorniae* 844, which is a double auxotrophic mutant for arginine and lysine. The two auxotrophic complement each other and the complementation selection mode was used in the identification of the fusants. The protoplasts of the two strains were prepared using Novozyme and fused in presence of 50% (w/v) polyethylene glycol. The fusants were regenerated in minimal medium. The fusion efficiency was 1.4%. The fusants showed 100% stability. To confirm the fusion, the DNA contents of the two double auxotrophic mutants and the fusant were estimated. DNA contents of *C. eichhorniae* 844, 822 mutant and the fusant were 0.71, 0.77 and 1.55  $\mu\text{g/g}$  cell respectively. These results show that the number of gene copies have been doubled in the fusant and their expression would yield higher primary and secondary metabolites.