PROTOPLAST REGENERATION AND TRANSFORMATION SYSTEM OF THERMOPHILIC FUNGUS *CEPHALOSPORIUM EICHHORNIAE*

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ABSTRACT

Genetic improvement of *C. eichhorniae* 152 (ATCC 38255), a thermophilic , obligately acidophilic and amylolytic filamentous fungus has been limited due to the low production and regeneration efficiency of the fungal protoplasts (19). In this study, high yields of protoplasts (10^8 per ml) were obtained by using Novozym 234 and pectinase as cell-wall lytic enzymes. The addition of the latter doubled the number of protoplasts being generated. Regeneration efficiency of *C. eichhorniae* was significantly improved by the isolation of mutants that could tolerate high osmotic stress in the medium. One of these mutants, S-6 was able to grow on medium containing either one of the following osmotic stabilizers: O.6 M NaCl, 0.6 M KCl, 0.8 M mannitol, 0.8 M glucose or 0.8 M sorbitol. Wild type strain was unable to grow on these media. The regeneration efficiency of S-6 protoplasts was 0.6, <0.1, 7.6, 7.6 and 7.6% on the aforementioned media, respectively. Wild type protoplasts could only be regenerated on media containing the sugar osmotic stabilizers with the efficiency of approximately 0.5-1% Protoplasts of S-6 mutant regenerated in a mycelium form on the regeneration agar plate whereas those of the wild type regenerated in a protoplast form on the same medium.

Transformations of both wild type and S-6 strains with plasmids, pOBT and pDH25, which carried selectable markers including phleomycin resistance gene and hygromycin resistance gene, respectively, gave no transformants whose digested chromosomal DNA would hybridize to either biotinylated probes, made of those plasmids by nick translation system. Optimization of transformation procedures did not alter the results. Transformation of A_2-57 with plasmid pSal23 carrying ornithine carbamoyl transferase gene (*argB*) resulted in 81, 189, and 72 transformants when the concentrations of the plasmid used was 6, 12 and 40 μg respectively. Transformation efficiency was calculated to be 15 transformants/μg plasmid DNA. However, there were no signals obtained on the digested chromosomal DNA of the transformants either detected by nonradioactive or radioactive labelling systems. It was believed that transformation occurred through the mechanism of gene conversion. The data reported here suggesting that the popularly used mesophilic *Aspergillus nidulans* based expression signals on those vectors did not function in this thermophilic fungus.