

VARANGKANA INTHARASEN: STRAIN IMPROVEMENT OF GLUCOSE ISOMERASE  
PRODUCING STREPTOMYCES SP.190-1 BY PROTOPLAST FUSION WITH XYLANASE  
PRODUCING STREPTOMYCES SP.42-9. THESIS ADVISOR : ASSO.PROF. PAIROH  
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Strain improvement of Streptomyces sp.190-1, an organism capable to produce glucose isomerase at a high level of 1200 units per gram of dry cells, has been carried out in order to generate xylanase by protoplast fusion with xylanase producing Streptomyces sp.42-9. Since the production of glucose isomerase required xylose, if Streptomyces sp.190-1 could produce xylanase, it would then be able to grow in medium with xylan containing material by hydrolyzing xylan to xylose which subsequently acted as inducer for glucose isomerase production.

Optimal conditions for formation and regeneration of protoplasts were investigated. Addition of glycine to the culture medium had no effect on protoplast formation of both species but markedly reduced cell growth. Age of mycelium also had no effect on protoplast formation, however, affected the regeneration of protoplasts. The mycelial age of 50-55 hours was optimum for protoplast regeneration in both species. With this mycelial age, the protoplast regeneration frequencies of 12.83 % and 1.60 % were obtained with Streptomyces sp. 190-1 and Streptomyces sp. 42-9, respectively.

Recombination frequencies obtained through protoplast fusion and conjugation between Streptomyces sp.190-1 and Streptomyces sp.42-9 were  $1.46 \times 10^{-3}$  and  $4.54 \times 10^{-3}$ , respectively by using their resistance to tetracycline in combination with ampicillin as markers. Higher recombination frequencies which were  $4.14 \times 10^{-2}$  for protoplast fusion and  $8.41 \times 10^{-5}$  for conjugation of Streptomyces sp.190-1 with Streptomyces sp.42-9 harboring plasmid pIJ4027 were obtained by using resistant ability to tetracycline and erythromycin for recombinant selection.

Twenty recombinants from 60 randomly selected recombinants could produce glucose isomerase at the level ranging from 600-900 units per gram of dry cells. They could produce xylanase in the range of 0.30 - 1.04 units per ml. when grown in a medium containing defatted rice bran and 3.97 - 8.64 units per ml. when grown in a medium containing xylan.