Part one

Production of Heterologous Protein from Methylotrophic Yeast, Pichia pasteris

Hiroyuki Uehara, Enoch YS Park, Mitsuyasu Okabe,

DaeYewn Shin*, DuBok Choi*,

Biotechnology Laboratory, Dept. of Applied Biological Chemistry, Shizuoka Univ.

Ohya, Japan

*Dept. of Environmental Engineering, Chosun Univ., Gwangju, Korea

Abstract

The expression of Heterologous protein (-amylase) in the methylotrophic yeast, P. pastoris was investigated. The mouse -amylase activity was inserted into the multi-cloning site of a Pichia expression vector, pPIC9, yielding a new expression vector pME624. The plasmid pME624 digested with Sa/I or Bg/II, and was introduced into P. patoris strain GS115 by the PEG 1000 method. Fifty-three transformants were obtained by the transplacement of pME624 digested with Sa/I or Bg/II into the HIS4 locus or into the AOX1 locus. Southern blot was carried out 11 transformants, which showed that mouse -amylase gene was integrated into the P. patoris chromosome. The optimizations of culture conditions were carried out for high-level expression of foreign protein in flask. An initial methanol concentration of 30g/l gave the highest -amylase activity. Especially, when the inoculation at 18 hr of the culture was carried out, the maximum -amylase activity was obtained, 260 U/ml. Using optimum culture conditions, fed-batch cultures in the air-lift bioreactor were carried out. The methanol consumption was increased with the increase of the culture time up to 4 days of culture, but after 4 days of culture it began to decrease, and finally, it hardly consumed up to the end of culture. The maximum -amylase activity was 720 U/ml at 3 days of culture when the cell concentration reached OD 95.