Molecular cloning and production of recombinant phytase from bacillus subtilis ASUIA243 in Pichia Pastoris.

ABSTRACT

Phytase gene obtained from Bacillus subtilis ASUIA243 was cloned into a medium vector and transformed into E. coli. Restriction enzyme digestion was conducted to get blunt-ended phytase gene and ligated into the Pichia expression vector, pPICZ�A. The recombinant vector, pPICZ�A-243HPp was then linearized with PmeI and transformed into P. pastoris strain X33. Screening for multi copy gene number of transformants was done by re-plating the selected colonies on increasing concentration of zeocin. One positive clone, X243HPp#2 was then grown in BMGY media as the starting culture, followed by induction in BMMY media for protein expression study. The supernatant was then analysed by SDS-PAGE and Western blot method to check the protein expression.

Keyword: Phytase; Bacillus subtilis; Pichia pastoris; Gene cloning.