Abstract

Keratinolytic alkaline proteases from microbial sources have considerable potential in biotreatments particularly for dehairing and bating, and removing substances like feathers that cause environmental pollution. The production of keratinase from this isolate is simple and agreeable to scale up. The organism grows on simple media with feathers as a sole carbon, nitrogen and energy source allowing its production from a cheap substrate and a commercial potential with low production cost.

A protease producing bacterial culture ‘S7’ (Code given by CBT, JNTU) was isolated from poultry waste samples, Hyderabad, India. It was related to Streptomyces sp. and resulted to be a new species of Streptomyces on the basis of biochemical properties and 16S r RNA gene sequencing. Cultural characters identified it as a mesophile secreting keratinase from 15°C-30°C. A two step Response Surface Methodology study was conducted for the optimization of keratinase production and enzyme activity from poultry feather by Streptomyces sp7. Initially different combination of salts was screened for the maximal production of keratinase at a constant pH of 6.5 and feather meal concentration of 5 g/l. The combination of K2HPO4, KH2PO4, NaCl, gave a maximum yield of keratinase (70.9 U/ml) production. In the first step of RSM study, the selected five variables (feather meal, K2HPO4, KH2PO4, NaCl and pH) were optimized by a 2³ full factorial rotatable central composite design that resulted in 95 U/ml of keratinase production. The results of ANOVA and regression of a second order model showed that the linear effects of feather
meal concentration (p<0.005) and NaCl (p<0.029), and interactive effects of all variables are more significant and values of quadratic effects of feather meal, (p<1.72e-5); K2HPO4, (p<4.731e-6); KH2PO4, (p<1.01e-10); and pH, (p<7.63e-7) are more significant than the linear and interactive effects of process variables. In the second step RSM studies a 2^3 rotatable full factorial central composite design and response surface analysis was done for the selection of optimal process parameters viz. pH, temperature and rpm on keratinase enzyme activity. These experiments yielded conditions of pH (11), temperature (45 °C) and rpm (300) for optimal keratinase activity. Purification of culture supernatant on sephacryl S-100 column indicated a keratinase with 67 % recovery, 2.5 fold purification and an estimated molecular mass of ~44,000 Da. Keratinase activity increased substantially in presence of Ca^{2+} and was inhibited in presence of PMSF and EDTA identifying it as a serine metalloprotease. Degradation of wide range of substrates (BSA, casein, gelatin, and starch) and stability in presence of detergents, surfactants and solvents make this keratinase extremely useful for biotechnological process involving keratin hydrolysis or in the leather industry.