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Full Length Research Paper

Statistical optimization of cultural conditions for chitinase production from fish scales waste by *Aspergillus terreus*

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Aspergillus terreus*, a local isolate from fishery polluted soil, was used successfully for the biodegradation of parrot fish-scales waste in favor of the production of highly active chitinase enzyme. Chitinase production was noticeably influenced by the culture medium and the highest enzyme production was attained through the acceleration growth phase (96 h). Pronounced decrease in chitinase production was concomitant with the sizes of fish-scales; larger sizes (normal, non-grinded) were the best for chitinase production than the finest grinded scales. Stagnant culture conditions were more favorite for chitinase production than shaked culture. Statistically based experimental designs were applied to optimize the production of chitinase by *A. terreus*. Eleven culture parameters were examined for their significance as effectors of chitinase expression using the Plackett-Burman factorial design. Concentrations of $FeSO_4.7H_2O$, glucose and $MnSO_4.2H_2O$ were the most significant factors affecting the process of enzyme production. The second optimization step was to figure out the levels of these three independent variables that generate maximum chitinase activity, using the Box-Behnken design. Maximum enzyme activity (4.309 u/min), which is approximately 1.81 folds the activity expressed in the basal medium, has been assayed at concentrations (g/l): $FeSO_4.7H_2O$ (9.5), glucose (6.5) and $MnSO_4.2H_2O$ (4.7), after 90 h of fermentation. A verification experiment was accomplished and revealed approximately 99% model validity. The crude chitinase was characterized and maximum activity was obtained in reaction mixture of 50°C incubation temperature, 2 ml crude enzyme, 0.5 ml of 10% colloidal chitin, pH 6 and reaction time of 10 min. The enzyme is thermostable and lost only less than 10% of its activity when heated at 60°C for 60 min. The effect of metal ions in enzyme activity revealed that the enzyme have specific requirement of Cu, Ca, Zn and Mn ions for its activity.

Key words: Chitinases, *Aspergillus terreus*, fish-scales, statistical optimization.