Optimal production of N-acetylchitooligosaccharides by bacillus sp. DYU-Too17

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ABSTRACT

In this study, a strain, Bacillus sp. DYU-Too 17, was isolated from Dacun Village in Changhua County. The aim of this study was to investigate an optimal condition for the production of N-acetylchitopentose by Bacillus sp. DYU-Too 17. In addition, the chitinase produced by this strain was purified and characterized. The one-factor-at a time method was first used to investigate the effects of carbon and nitrogen sources on the production of N-acetylchitopentose. When α-chitin was the sole carbon source, the major product was N-acetylchitopentose. Especially, the highest production of N-acetylchitopentose (0.186 g/L) was obtained in a medium of 4% α-chitin; NH4Cl seemed to be a better nitrogen source to produce N-acetylchitopentose, and the concentration was 0.127 g/L in a medium containing 0.3 g/L NH4Cl; The highest yield of N-acetylchitopentose (0.080 g/L) was obtained at 30℃. The crude enzyme was obtained from a culture of Bacillus sp. DYU-Too 17 in a medium containing 4% α-chitin and 0.3 g/L NH4Cl at 30℃. The purification procedures included precipitation by ammonium sulfate, dialysis, and anion exchange chromatograph (DEAE-Sepharose CL-6B). The optimal reaction temperature for the purified chitinase was 40℃, and the optimal reaction pH was 7.0. The purified chitinase was stable at 10~30℃ and pH 5-8. Metal ions Fe3+, Hg2+, Mg2+ and Zn2+ could inhibit the chitinase activity, and however, Ag+ could enhance the activity. The molecular weight of the major chitinase was determined to be 36 KDa, and its kinetic constants Km and Vmax were 1.7 g/L and 333 U/L, respectively. In addition, response surface methodology was used to search for an optimal condition for culturing Bacillus sp. DYU-Too 17. From the analysis of variance, the α-chitin concentration was significant for the N-acetylchitopentose production ($p<0.05$). The optimum concentrations of α-chitin and NH4Cl for the N-acetylchitopentose production were determined to be 4.47% and 0.36 g/L, respectively. The predicted maximum production of N-acetylchitopentose was 0.367 g/L. In addition, comparison between the actual observations (experimental data) and predicted response showed no significant differences. This indicates that the response surface model could well predict the N-acetylchitopentose production of Bacillus sp. DYU-Too 17.

Keywords : Chitinase、N-Acetylchitopentose、Response surface methodology、Optimum condition、Purification and characterization

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