The Studies on the chitinase of Bacillus subtilis W-118

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ABSTRACT

The fermentation broth of the chitinase bacterium Bacillus subtilis W-118 , was isolated from soil in the northern Taiwan. Under the optimized culture condition that the culture was shaken in 250 mL flasks with 100 mL medium （3% shrimp and crab shell powder, 0.1%K2HPO4, 0.05%MgSO4 , pH 6.0 adjusted by phosphate buffer solution）at 30℃for 3 days. The chitinase was purified from the culture supernatant of W-118 by ammonium sulfate fractionation , DEAE-Sepharose CL-6B, Sephacryl S-200 gel permeation chromatography and chromatofocusing. The purified enzyme estimated by protein 200 chip have a molecular weight of 20.6 kDa. After separation and purification, the activity of the chitinase was still stable at 50°Cand pH 5～7, while the optimum temperature and pH for the enzyme reaction were at 37°Cand pH 6. The mian purpose of this thesis is to investigate the hydrolysis of colloiadal chitin by Bacillus subtilis W-118 to produce N-acetylchitooligosaccharides with low degrees of polymerization (DP) from 1 to 6. The best analytical N-acetylchitooligosaccharides (NACOs) is reverse phase High Performance Liquid Chromatography. It was found that the optimum temperature and reaction time for production of NACOs were 37℃ and 1 hour. Longer reaction time lead to the generation of NACOs with lower DP's. The N-acetylchitooligosaccharides examined for their growth inhibition effects in human leukemia cell lines K562 cells were treated with these compounds for 24, 48, 72 hours and their proliferation was determined by WST-1 reagent. N-acetylchitooligosaccharides had the highest growth inhibitory, and morphological changed. The effect of N-acetylchitooligosaccharides on hyphal growth, was investizated. The result showed that N-acetylchitooligosaccharides caused abnormal hyphal fragmention on the Fusarium oxysporum.

Keywords : Bacillus subtilis 、chitinase、SCSP、K562、N-acetylchitooligosaccharides

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