# Purification and characterization of chitinase produced by indeqineous Aeromonas sp.


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**ABSTRACT**

In this study, 11 strains were screened from soil all over Taiwan to produce chitinases. Among them, strains DN15 and DN23 produced higher level of reducing sugars. According to the DNA sequences of these two strains identified by the NCBI (National Center for Biotechnology Information), Strains DN15 and DN23 were then named as Aeromonas hydrophila DYU-Too15 and Aeromonas punctata DYU-Too16. The α-chitin content and nitrogen species in the CB (chitin broth) media were altered to search for a better culture condition to produce N-acetyloligosaccharides. In a CB medium, when the content of α-chitin was altered, there was no effect on the variety of N-acetyloligosaccharides produced by A. hydrophila DYU-Too15 or A. punctata DYU-Too16, but the content of chitooligosaccharides increased with the increase of the α-chitin concentration. If the medium contained 4% α-chitin, the highest amount of N-acetylglucosamine (about 3.65 g/L) was produced by A. hydrophila DYU-Too15 at 96 h. When the medium contained 5% α-chitin, N-acetylchitotriose was the major product and reached about 1.22 g/L at 96 h. There was no effect on the variety of N-acetyloligosaccharides produced by A. hydrophila DYU-Too15 in a CB medium with various nitrogen sources (yeast extract, peptone, tryptone, yeast extract + peptone, NH4Cl). The major product was N-acetylglucosamine. For A. punctata DYU-Too16 in a CB medium with yeast extract and peptone as the nitrogen sources, the major products included N-acetylglucosamine and N-acetylchitotriose. However, the only major product was N-acetylglucosamine if each of the other four nitrogen sources was used as the nitrogen source in the CB medium. In order to obtain N-acetylchitooligosaccharides, the newly screened two strains and the early obtained strain, Aeromonas hydrophila DYU-Too14, in our laboratory were examined. A. hydrophila DYU-Too14 can produce N-acetylchitopentaose and N-acetylchitohexaose in a CB medium by using NH4Cl as a nitrogen source. Since N-acetylchitopentaose and N-acetylchitohexaose can enhance the immune system, inhibit tumor cell growth and possess other physiological activities, and their values are much higher than N-acetylglucosamine and N-acetylchitotriose produced by the other two strains. Thereafter, A. hydrophila DYU-Too14 was used as the target strain for chitinase purification. To separate the chitinase produced by A. hydrophila DYU-Too14, this strain was cultivated in a CB medium containing 4% α-chitin as the carbon source and 0.7 g/L NH4Cl as the nitrogen source. The supernatant of the culture containing crude enzyme was first precipitated by ammonium sulfate, and then the precipitate was further purified through dialysis, anion gel (DEAE-Sepharose) chromatography. From DEAE-Sepharose gel chromatographic diagram, two peaks of Fractions 90-93 and 94-98 possessed chitinase activity. Hence, the above chitinase was used to hydrolyze colloidal chitin solution, the hydrolysates were separated through centrifuge and lyophilization, and its composition was analyzed by HPLC. The hydrolysates contained N-acetylchitopentaose and N-acetylchitohexaose. Through electrophoresis, the molecular weight of the chitinase was identified to be 25 kDa.



Keywords : chitinase、Aeromonas hydrophila DYU-Too14、N-acetylglucosamine、N-acetylchitotriose、N-acetylchitopentaose、N-acetylchitohexaose


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