# Isolation of an N-acetylchitooligosaccharides Producing Bacterium and Characterization of Its Chitinases


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**ABSTRACT**

In this study, a strain, named as DYU-Too12, was isolated from the soil of Xin-bao in Changhua County to degrade chitin into N-acetylchitooligosaccharides, and the culture conditions were also examined. This strain was identified to be a Gram ’s negative rod shaped bacterium. The strain was cultivated separately in a 5-L batch fermentor in a CB medium with different levels of initial dissolved oxygen (100% and 40% saturation). The highest activities of chitinase were 833 U/L at 48 h and 933 U/L at 60 h for 100 % and 40% saturation, respectively. N-acetyl-chitooligosaccharides with DP (degree of polymerization) 1~4 were produced in these two cases, and highest yields of N-acetyl-glucosamine were 3.06 and 0.1 g/L, N-acetyl-chitobiose was 1.0 and 0.41 g/L, N-acetyl-chitotriose were 0.22 and 0.40 g/L, N-acetyl-chitotetraose were 0.19 and 1.03 g/L, respectively.
N-acetyl-chitooligosaccharides with higher DP were produced by strain DYU-Too12 when cultivated at a lower level of dissolved oxygen. The strain was cultivated separately in a 500-mL flask with each of a volume of 100 or 200 mL of the CB medium. The supernatant of the culture of strain DYU-Too12 cultivated in 100 mL at 72 h and 200 mL at 96 h were further purified. Purification of chitinases was carried out by protein precipitation with ammonium sulfate, dialysis, anion exchange of DEAE-Sepharose CL-6B and gel filtration of Sephacryl S-100 HR. A peak showing chitinase activity was observed, and fractions of this peak were collected for further analysis. The chitinases were purified by 3.8 and 2.77 fold, and the specific activities were 4.7 and 4.02 U /mg protein, and the yields of chitinases were 21.8% and 7.23%, respectively. The optimum reacting temperature was 40℃, and the optimum pH was 6.0 for the chitinases. Metal ions such as Hg2+, Zn2+, Ag+ and Mn2+showed an inhibitive effect on the chitinase activity and EDTA could enhanced the activity.



Keywords : N-acetyl-chitooligosaccharides, chitinase, isolation and purification of enzyme


**Table of Contents**

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