Screening of Microbes to Produce N-Acetylchitooligosaccharides and Purification of the Chitinases

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ABSTRACT

N-acetylchitooligosaccharides have antitumor, immunostimulating and antibacterial functions, and therefore, are widely used in many fields. However, due to the limitation of preparation methods, the cost of production still remains high. Present methods for producing N-acetylchitooligosaccharides have advantages and drawbacks. In this study, microbial fermentation to produce N-acetylchitooligosaccharides was investigated due to its low pollution and specific action of enzyme. A microbe, isolated from Shui-Wei Creek in the Miaoli County and named as DYU-Too 11, was used in this study to degrade chitin. The microbe had been identified to be Aeromonas hydrophila by the Food Industry Research Development Institute in Hsin-Chu, Taiwan, and was named as Aeromonas hydrophila DYU-Too 11. Two carbon sources, chitin and colloidal chitin, were used to explore the difference of N-acetylchitooligosaccharides and chitinase produced by this microbe. For the case of using chitin as a carbon source, N-acetyl-glucosamine and N-acetylchitooligosaccharides with DP (degree of polymerization) 2 ~ 5 were produced, and their highest yields were 0.79, 0.94, 0.40, 0.16 and 0.26 g/L, respectively. The activity of crude chitinases was 370 U/L. For the colloidal chitin case, N-acetylglucosamine and N-acetylchitooligosaccharides with DP 3 ~ 4 were produced, and their highest yields were 15.8, 0.37 and 0.06 g/L, respectively. The activity of crude chitinases was 310.4 U/L. Therefore, chitin seemed to be a better carbon source for producing N-acetylchitooligosaccharides. To investigate the effect of chitin concentration on the product, Aeromonas hydrophila DYU-Too 11 was cultivated in media with various chitin concentrations. N-acetylglucosamine and N-acetyl- chitooligosaccharides with DP 2, 4 and 5 were produced in the culture of 1% chitin, meanwhile, N-acetylglucosamine and N-acetylchitooligosaccharides with DP 2 ~ 6 were produced in the 2% chitin medium. For higher chitin contents, e.g., 3%, 4% and 5%, N-acetylchitooligosaccharides with higher DP would be obtained. Therefore, selection of chitin concentration should depend on what target hydrolysates wanted. In order to isolate chitinases secreted by Aeromonas hydrophila DYU-Too 11, the protein in the culture supernatant was precipitated with ammonium sulfate of 0 ~ 80% saturation. The precipitate was re-dissolved in a 50 mM Tris-HCl buffer (pH 7.8), and then purified through DEAE-Sepharose CL-6B and Sephacryl S-100. A peak showing chitinase activity was observed, and fractions of this peak were collected for further analysis. Molecular weights of the chitinases were identified to be 60 and 43 kDa by Zymogram analysis. The optimal reacting temperature of the chitinases was 40°C, and the optimal reacting pH was 8.0. The chitinases were stable in the range of 10 ~ 40°C and still retained 70% of the original activity, while at 50 °C only 13.8% activity left. The chitinases were stable in the range of pH 6 ~ 9 and retained 50% activity. The effect of metal ions on the chitinase activity was also investigated. Results showed that Ca2+、Zn2+、Mn2+ and Hg2+ions have an inhibitive effect on the chitinases, especially the inhibition of Zn2+ was very substantial, only 6.5% activity left.

Keywords : chitin ; N-acetylchitooligosaccharides ; chitinase ; isolation and purification of enzyme

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