Production of N-acetylchitooligosaccharides from Chitin by Indigenous Strains

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ABSTRACT

Abstract Recently, studies on physiological functions of N-acetylchito- oligosaccharides have brought attention from researchers in the fields of food science and medicine. N-acetylchitooligosaccha- rides are important for their general immune system enhancement, including infection prevention, anti-tumor, anti-fungi and anti- bacteria capabilities, etc. The main purposes of this study are (1) to screen some strains producing acetylchitooligosaccharides with higher degrees of polymerization from local soil, and (2) to produce N-acetyl- chitooligosaccharides in a large quantity by a fermenter. Meanwhile, an optimal operating condition for bacterial growth and the production of N-acetylchitooligosaccharides was explored. Eight indigenous strains named sequentially from “ Too 1 ” to “ Too 8 ” were isolated from soil and ocean sand to produce N-acetylchitooligosaccharides. Batches of each of these eight strains were grown in flasks to compare their chitinase activities. Strain “ Too 3 ” has been shown to yield the most active chitinase. While N-acetylchito-hexaose (GlcNAC)6 can be obtained from the hydrolysis of chitin with chitinases produced by “ Too 7 ” and “ Too 8 ”, only (GlcNAC)2 was found in the hydrolysates of chitin from strain “ Too 2 ”. “ Too 7 ” would reach an exponential phase in 8 h and a stationary phase within around 16 h if cultivated in a colloidal chitin broth in a rotary shaker. The pH level was raised during the microbial growth period, possibly caused by the ammonia produced by the N-acetylchitooligosaccharide metabolism. Furthermore, “ Too 7 ” was cultivated in CB (chitin broth), CCB (colloidal chitin broth), and GB (glucose broth) separately to compare their chitinase activities. The chitinase produced in CB has been shown to be the most active (192.56 U/g), that in CCB (184.21 U/g) being the second, and GB (80 U/g) the least. The experimental results showed that “ Too 7 ” needed chitin to induce the production of chitinase. “ Too 7 ” could produce more chitinase, when it cultivated in basic medium with extra inorganic nitrogen. Though “ Too 7 ” could directly use ammonia, only inorganic nitrogen source seems not sufficient for good growth of this strain. “ Too 7 ” was cultivated in a fermenter using either CB or LPB as a medium. The experimental results showed that in either CB or LPB, the hydrolysates of chitin consisted mainly of N-acetlyglucosamine through the exponential phase, but N-acetylchitooligosaccharides with higher degrees were also detected. Perhaps the N-acetylchitooligosaccharides are the intermediate products of hydrolysis in the chitin metabolism. In addition, the amount of N-acetylchito-biose increases as the increase of the incubation time in both CB and LPB. N-acetylchitobiose was produced more in LPB (0.27 g/L) and only 0.16 g/L in CB. It is possible that N-acetylchito oligosaccharides with higher degrees can be produced by less biomass and less chitinase, and consequently, the hydrolysis of chitin decreases. Therefore, if cultivation conditions such as incubation time, hydrolysis rate, biomass and substrate concentrations are properly controlled, N-acetylchitooligosaccharides with higher degrees would be obtained. Keywords: chitin, chitinase, N-acetylchitooligosaccharides

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