Chitin-deacetylase production by microorganisms: a key step towards a sustainable production of bioactive chitooligosaccharides from marine shell wastes

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The marine processing industry generates more than 20 million tonnes/year of waste. This highly perishable material can represent up to 45% of shellfish weight, including heads, thorax, claws, and shells. Once extracted from proteins and minerals present in the shells, during two processes called demineralisation and deproteinisation, chitin can be chemically converted into chitosan (deacetylation), which in turn can be depolymerized into fragments of lower molecular weight, called chitooligosaccharides (COS). COS are a group of bioactive molecules with many applications in food, pharmaceutical and medical industries. Among the four steps needed to transform chitin from marine shells into COS, deacetylation is considered the most challenging. Chitin deacetylases (CDAs) are still too expensive commercially and their biotechnological production is still limited due to low extracellular production by most microorganisms. Although many efforts have been made in the last two decades, this is considered a key step in obtaining high-quality COS that exhibit specific biological properties. We tested Penicillium chrysogenum (ATCC 10106) as a biotechnological producer of intra and extracellular CDA. For this, static solid-state fermentation (SSF) using wheat bran and static liquid fermentation were tested at lab scale (200 mL), for 10 days. The extraction of the extracellular enzyme was done by mixing the fermentation substrate with buffer solution, followed by centrifugation, while the intracellular enzyme was recovered by the collection of spores followed by one of two methods: (1) beads milling at a mass ratio of 1:2 (spores:glass beads) or (2) manual grinding followed by US bath for 15 min. Extracted solutions were tested for CDA enzymatic activity determined by the 4-nitroacetanilide reaction. Appropriate inactivated enzyme blanks were tested for each condition. Although extracellular production of CDA was not detected by both methods (SSF and liquid), intracellular production of CDA by P. chrysogenum was high for both extraction methods, but beads milling resulted in a 2-fold higher enzymatic activity (237.9 U/g of protein). Our results indicate that P. chrysogenum is an interesting producer of intracellular CDA. Chitin deacetylation using P. chrysogenum’s enzyme can produce COS from a high pollutant waste, with the quality required to express COS health-promoting properties.