



Review

Recent developments in chitosanase research and its biotechnological applications: A review



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ABSTRACT

Chitosanases (EC 3.2.1.132) are glycosyl hydrolases that catalyse the endohydrolysis of β -1,4-glycosidic bonds of partially acetylated chitosan to release chitosan oligosaccharides (COS). Chitosanases are isolated, purified and characterised from different sources mainly from bacteria and fungi. Chitosanases have received much attention due to their wide range of applications including the preparation of bioactive COS and fungal protoplasts, as biocontrol agent against pathogenic fungi and insects, the bioconversion of chitinous bio waste associated with seafood processing, etc. Bioactive COS produced by the enzymatic hydrolysis of chitosan have finds numerous health benefits as well as other biological activities. This review summarizes the recent advances in chitosanases research, the enzyme production processes, characterization, genetic improvement and their applications.

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Contents

| | |
|---------------------------------------------------|-----|
| 1. Introduction | 393 |
| 2. Occurrence and distribution of chitosanases | 393 |
| 2.1. Bacterial chitosanase | 393 |
| 2.2. Fungal chitosanases | 393 |
| 2.3. Cyanobacterial chitosanase | 394 |
| 2.4. Plant chitosanases | 394 |
| 3. Definition and classification of chitosanases | 394 |
| 4. Fermentative production of chitosanase | 394 |
| 5. Measurement of chitosanase activity | 395 |
| 6. Purification of microbial chitosanases | 395 |
| 7. Basic biochemical properties of chitosanases | 395 |
| 7.1. Molecular mass | 395 |
| 7.2. pH and temperature optima | 396 |
| 7.3. Enzymatic hydrolysis | 396 |
| 7.4. Inducers of chitosanase synthesis | 396 |
| 7.5. Effect of metal ions on chitosanase activity | 397 |
| 7.6. Enzyme structure | 397 |
| 8. Immobilised chitosanase | 397 |
| 9. Cloning and genetic improvement | 397 |
| 10. Biological roles of chitosanase | 398 |
| 11. Potential applications | 398 |
| 12. Futuristic considerations | 399 |
| Acknowledgement | 399 |
| References | 399 |

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1. Introduction

Chitosan is a poly cationic natural polymer with a unique structure and functional properties. It is an unbranched copolymer consisting of β -(1 \rightarrow 4)-2-acetamido-D-glucose (*N*-acetyl-D-glucosamine, GlcNAc) and β -(1 \rightarrow 4)-2-amino-D-glucose (D-glucosamine, GlcN) units with the latter usually exceeding 80% and the GlcNAc and GlcN residues are randomly distributed and not blocked together (Zitouni et al., 2012) (Fig. 1). Chitosan is found in nature as a structural component mainly in the cell wall of Zygomycetes fungi and also found in the cell wall of Chlorophycean algae *Chlorella* sp. and in insect cuticle (Hsu, Chung, Chang, & Sung, 2012). Chitosan is the principal *N*-deacetylated derivative of chitin (a β -1,4 linked polymer of GlcNAc), although the *N*-deacetylation is almost never complete. Currently chitosan is produced commercially from crustacean's (shrimp and crab) chitin by *N*-deacetylation, to different degree using hot concentrated alkali. Thus, there is no clear distinction between chitin and chitosan based on the degree of *N*-deacetylation; the term chitosan represents a collective name for a family of deacetylated chitins at different degrees (Fig. 1). The studies of chitosan with respect to their preparation, structure, functional properties and applications have been extensively reviewed (Zitouni et al., 2012).

Chitosan is usually susceptible to a number of enzymes; these include specific (chitosanases) and non-specific (carbohydrases, proteases, lipases etc.) chitosan hydrolysing enzymes. Chitosanases have been generally recognised as enzymes that specifically hydrolyse chitosan but not chitin (Kim & Rajapakse, 2005). In 2004, the Enzyme nomenclature committee had defined chitosanases (EC 3.2.1.132, Chitosan *N*-acetylglucosaminohydrolase) as the enzyme capable of performing endohydrolysis of β -1,4-linkages between GlcN residues in a partly acetylated chitosan, from the reducing end (Fig. 2a). Later, in 2008, the committee created a new (second) class of enzyme, *exo*- β -D-glucosaminidase (EC 3.2.1.165) that attack chitosan from its non reducing end (Fig. 2b). In addition, some other non specific enzymes such as common carbohydrases, proteases and lipases have also shown their hydrolytic ability on chitosan (Kim & Rajapakse, 2005). As on date a large number of specific chitosanolytic enzymes have been reported from different microorganisms including bacteria, fungi and cyanobacteria and plants.

Recent researches on chitosanases have received much attention due to their wide range of applications in various fields. Practical applications of chitosanase include the preparation of bioactive COSs (Kim & Rajapakse, 2005; Ming, Kuroiwa, Ichikawa, Sato, & Mukataka, 2006), preparation of fungal protoplasts particularly for Zygomycetes, a biocontrol agent to increase the resistance of plants against pathogenic fungi (Hsu et al., 2012),

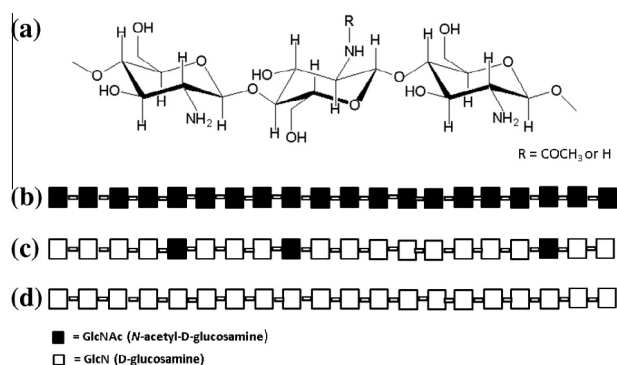


Fig. 1. Chemical structure of chitosan (a). Distribution of GlcNAc and GlcN in chitin (b), partially *N*-deacetylated chitosan (c) and fully *N*-deacetylated chitosan (d).

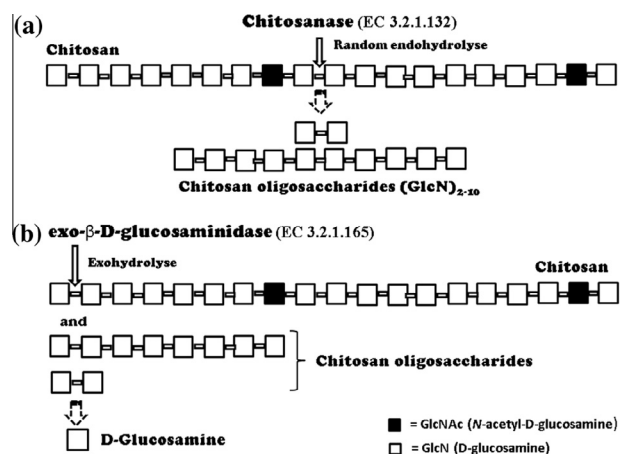


Fig. 2. Mode of action of chitosanase (a) and *exo*- β -D-glucosaminidase (b).

chitosan mediated gene delivery and the bioconversion of marine crustacean chitinous bio waste (Wang, Chao, Liang, & Chen, 2009; Wang, Tseng, & Liang, 2011). Chitosanase mediated hydrolysis has advantages over the chemical/physical mediated hydrolytic production of COS, in which chitosanases can catalyse the hydrolysis under mild reaction conditions and do not produce monosaccharides (Kim & Rajapakse, 2005; Ming et al., 2006). Reviews on chitosanases with respect to its production and application (Somashekar & Joseph, 1996) have been reported earlier. The present review will focus not only on the current knowledge of chitosanases, their occurrence and classification but also on microbial production, biochemical properties, genetic improvement and applications based on research specifically carried out in the last ten years.

2. Occurrence and distribution of chitosanases

Chitosanase is produced by microbes and plants, where they play an important role in nutrition and defence. Chitosanase was first described in 1973 from different soil microorganisms. Over the past 40 years, several research papers have been published on the occurrence, production, purification and characterization of chitosanase from different microorganisms including bacteria, fungi and cyanobacteria; and plants. Detailed literature is available on various microbial sources of chitosanase (Somashekar & Joseph, 1996).

2.1. Bacterial chitosanase

Extracellular chitosanases has been reported from several bacteria including *Bacillus* sp. (Gao, Ju, Jung, & Park, 2008; Wang et al., 2009; Zitouni et al., 2012), *Serratia* sp. (Wang, Peng, Liang, & Liu, 2008b), *Janthinobacterium* sp. (Johnsen, Hansen, & Stougaard, 2010), *Paenibacillus* sp. (Zitouni et al., 2012), *Acinetobacter* sp. (Wang et al., 2011) and *Streptomyces* sp. (Jiang, Chen, Chen, Yang, & Zou, 2012). Among these bacteria, species of *Bacillus* and *Streptomyces* were studied most extensively as a chitosanase source even up to structural and molecular level.

2.2. Fungal chitosanases

As compared to bacteria, there have been few reports on chitosanases from fungi. Chitosanase production has been reported in different fungi including *Aspergillus* sp. (Zhang, Sang, & Zhang, 2012), *Gongronella* sp. (Wang, Zhou, Yuan, & Wang, 2008a; Zhou,

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