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# Production of recombinant *Bacillus subtilis* chitosanase, suitable for biosynthesis of chitosan-oligosaccharides

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## HIGHLIGHTS

- Chitosanase (Csn) is used to convert chitosan from chitin waste to chitosan-oligosaccharides (COS).
- Csn from *B. subtilis* was cloned, efficiently expressed and secreted in *E. coli*.
- *B. subtilis* Csn thermostability is dependent on the substrate.
- Recombinant *B. subtilis* Csn is suitable for industrial application.
- Crude culture media containing secreted Csn was used to convert chitosan to COS.

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## ABSTRACT

Chitosanases are enzymes that catalyse the hydrolysis of the β-1,4 glycosidic bond of chitosan. One of the most promising applications of this enzyme is for the bioconversion of chitosan into value-added chitosan-oligosaccharides (COS). GH46 chitosanase (Csn) from *Bacillus subtilis* 168 was expressed in *Escherichia coli* by fusing the gene encoding mature Csn to the *E. coli* OmpA signal peptide sequence. The recombinant enzyme was secreted into the culture supernatant. The recombinant Csn showed high specific activity and stability over a wide range of pH. The enzyme was >100 times more thermostable in the presence of the substrate, with a half-life time of activity ($\tau_{1/2}$) of approximately 20 h at 50 °C and pH 5.5. Efficient bioconversion of chitosan into different mixtures of COS, using crude culture supernatant containing secreted enzyme was demonstrated.



## 1. Introduction

Chitosan *N*-acetylglucosaminohydrolase or chitosanases (EC 3.2.1.132) are enzymes that catalyse the hydrolysis of the β-1,4 glycosidic bond of chitosan, a partially deacetylated derivative of chitin, which comprises *N*-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) residues (Dahiya et al., 2006). These enzymes can be found in a wide variety of microorganisms including Gram-positive and Gram-negative bacteria, yeast and fungi (Eijsink et al., 2010). The enzymes belong to glycoside hydrolase (GH) families 5, 7, 8, 46, 75 and 80, according to the Carbohydrate-Active Enzymes database (CAZy) (Cantarel et al., 2009). While families GH5, GH7 and GH8 contain a few chitosanases and other glycoside hydrolases, specifically, families GH46, GH75 and GH80 comprise exclusively chitosanases (Eijsink et al., 2010). Chitosanase from family 46, especially those from *Streptomyces* (Dubeau et al., 2011; Fukamizo and Brzezinski, 1997) and *Bacillus circulans* (Fukamizo et al., 2005) are presently the best-studied enzymes.

One of the most important and promising applications of chitosanases is the bioconversion of chitosan into chitosan-oligosaccharides (COS) (Aam et al., 2010). These polysaccharide oligomers have been shown to have potential medical applications (Aam et al., 2010; Khoushab and Yamabhai, 2010), including in gene delivery, as drugs against asthma, malaria and cancer, antibacterial and antifungal agents, ingredients for wound dressing and bone strengthening, as well as substances for lowering serum glucose in diabetics (Aam et al., 2010; Khoushab and Yamabhai, 2010). The advantage of using chitosanase to produce COS over physical methods such as the use of mirowaves, γ-rays, ultrasonication, or hydrothermal treatments is the more environmentally friendly process that produces more defined COS mixtures.

Chitosan can be prepared from chitin by homogeneous or heterogeneous deacetylation, resulting in polysaccharide polymers with various degrees of N-acetylation (DA), polymerisations (DP), and molecular weight distributions (Aam et al., 2010). Since the various biological activities of COS are dependent on the degree of poly-

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