

# Antimicrobial Activity of Chitooligosaccharides



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## 1 Introduction

Chitin (Fig. 1) is a natural biopolymer polysaccharide distributed among the shells of sea arthropods. Every year approximately six to eight million tons of shell waste are produced by seafood industry due to its improper discard in environment. However, chitin is extracted at a commercial scale due to its vast applications aiding in reduction of marine waste (Yan and Chen 2015; Singh et al. 2021). The partial deacetylation of chitin generates an N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) copolymer known as chitosan (Simat et al. 2020) with low solubility in universal solution which makes its application difficult in the food and biomedical sectors. Moreover, high molecular mass, the pattern of acetylation and deacetylation degree are the few variables that acts as barriers for its extensive use in the biomedical field (Li and Zhuang 2020). Few mechanisms such as perturbation of ion transport on the microorganisms have been suggested for the antimicrobial property of chitosan (Yin et al. 2020).

But to overcome these boundaries, hydrolysis of chitosan has been performed yielding small, soluble chitooligosaccharides (COSs) with higher solubility and low viscosity as compared to chitosan along with several biological properties such as antimicrobial activity against a broad spectrum of food-borne microbes acknowledging it as a natural food preservative (Devlieghere et al. 2004; Silva et al. 2021). COSs (Fig. 2) are the chitosans with a degree of polymerization less than 20 and an average molecular weight less than 3.9 kDa. They can also be called as chitosan oligomers or chitooligomers (Lodhi et al. 2014). Due to their hydrophilic nature

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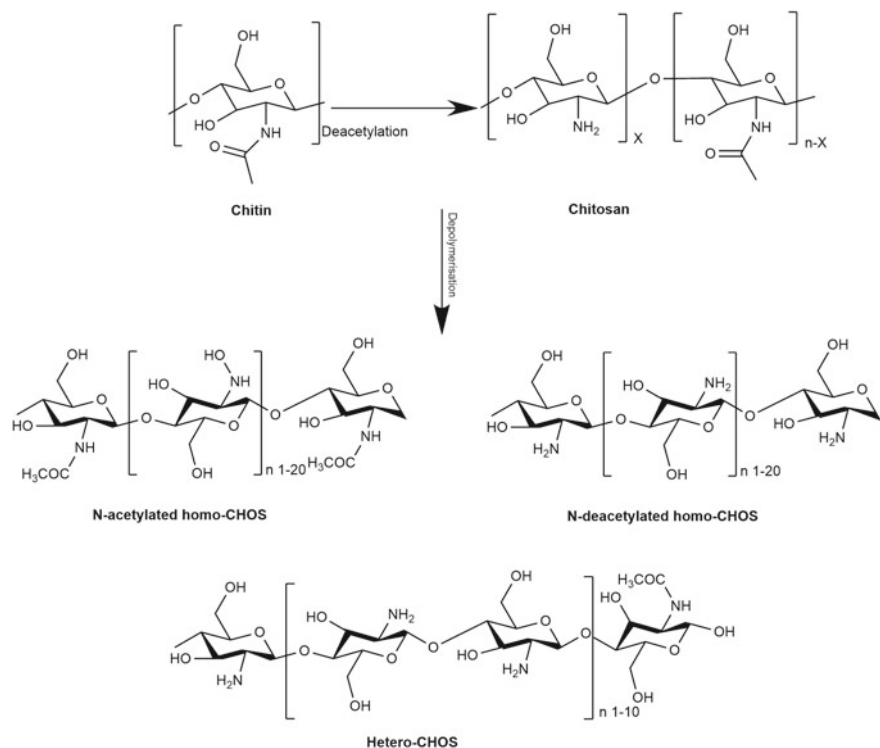
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**Fig. 1** Chemical structure of chitin and its derivatives (Jafari et al. 2020)

and low molecular weight, they are of greater demand than their precursor molecule chitosan (Liaqat and Eltem 2018). The shorter chain length and free amino groups in D-glucosamine units in COSs can be considered as a reason for their water solubility and lower viscosity (Bahrke 2008). In contrast to chitin and chitosan, COS has properties like cell membrane penetrability, easy absorption other than water solubility which makes it an extremely valuable product (Liaqat and Eltem 2018). Therefore, the enzyme-mediated production of chitoooligosaccharides has been done from chitosan. The glucosamine and acetylated glucosamine rings in COS are connected with each other by  $\beta$  1,4-glycosidic bond (El-Sayed et al. 2017). It is known that the principle characteristic factors directly affecting the physicochemical and biological activity of COS are the degree of polymerization and molecular weight. COS with a degree of polymerization six or higher and molecular weight low are more biologically active than the COS with low degree of polymerization and high molecular weight (Prashanth and Tharanathan 2007).

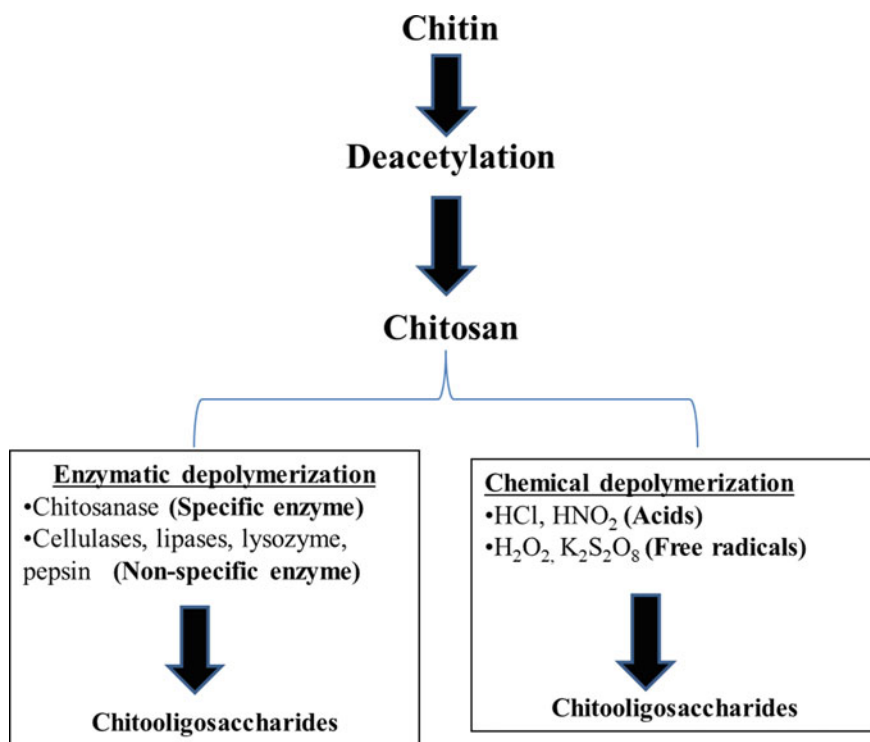


Fig. 2 Production of chitooligosaccharides by depolymerization of chitin

## 2 Antimicrobial Activity of Chitooligosaccharides

In 1979, the broad-spectrum antimicrobial activity of chitosan and its derivatives was reported by Allan and Hadwiger for the first-time (1979). Since then, chitosan and COS have been used by industries for their antimicrobial properties. To define the antimicrobial potential of COS and its utilization in agriculture, medical and food sectors, the use of well-characterized COS is necessary for in vitro as well as in vivo studies (Liaqat and Eltem 2018). Due to the antimicrobial resistance in various microbes, there is a need for new compounds with enhanced activity presenting options for the treatment of several bacterial, yeast and parasitic diseases (El-Sayed et al. 2017; Bloom and Cadarette 2019). The degree of polymerization, degree of N-acetylation and physicochemical characteristics, microorganism type are few factors responsible for the antimicrobial activities of COS (Chung et al. 2004). Moreover, the existence of the primary amino groups in the structure of COS is also supposed to be the reason for its antimicrobial property for now, since the molecular reason is not known yet. The alteration in cell membrane permeability by COS causes the death of the microbial cell due to the release of cell constituents and uncontrolled entry of materials into the cell from outer environment (Choi et al.

2001; Mei et al. 2015). Through the use of UF membrane in association with enzymatic reactor, three different chitooligosaccharides fractions were prepared based on their molecular weight (Jeon and Kim 2000). These were used to determine the antimicrobial activity against Gram-positive, negative and lactic acid bacteria in order to explore the effects of molecular mass on the microbial population (Silva et al. 2021). COS was observed to be highly effective for *S. typhi* that was inhibited 90% (approximately) at 0.1% concentration with 0.12% MIC (Minimum Inhibitory Concentration). For *E. coli* and *E. coli* O-157, high molecular weight COS possessed the highest antibacterial effect with MIC 0.06 and 0.12%, respectively. All COSs indicated a suppressed growth for *Pseudomonas aeruginosa* with antimicrobial activity 50% or lower at 0.1% concentration. The COS with higher molecular weight (>10 KDa) showed effective activity against pathogens rather than non-pathogens (Jeon et al. 2001). An enzymatically produced, well-characterized COS has been reported for its in vivo anti-dermatophytic activity against *Trichophyton rubrum* in a guinea pig model indicating clinical application of COS due to its safety, biocompatibility and biodegradability (Mei et al. 2015). Antifungal effect of COS was also determined against the *Botrytis cinerea* in vitro and in vivo condition with a combination of synthetic fungicide and COS. Results rebelled that combination of both was highly effective against the *Botrytis cinerea* (Rahman et al. 2015).

El-Sayed and co-workers reported the in vitro antimicrobial activity of four sets of chitooligosaccharides (I, II, III and IV) were prepared using immobilized pepper chitosanase with a molecular weight greater than 100, 100–10, 10–1 and less than 1 KDa, respectively. For antimicrobial activity determination, disc diffusion plates method (50 µg/ml) was used to detect in vitro antimicrobial activity against the pathogens namely *B. cereus*, *B. subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces chevalieri* in comparison to chitosan. Results showed that immobilized pepper chitosanase can be helpful for the industrial production of antimicrobial chitooligosaccharides from chitosan (El-Sayed et al. 2017). The combination of COS in chitosan films was used to improve the antimicrobial activity against the pathogens such as *Lactobacillus plantarum*, *S. aureus*, *B. cereus Serratia liquefaciens* and *E. coli* (Fernández-de Castro et al. 2016). This serves as a proof of the fruitful use of COS in food packaging films due to their antimicrobial activities. The white-leg shrimp shells have also been used for the extraction of chitosan and COS through chemical treatment to determine their antibacterial and antifungal activities against several bacterial and fungal species (spoilage and pathogenic microbes). COS was highly soluble in water than chitosan due to its low molecular weight and high degree of deacetylation (13 kDa and 54.83%). The marked inhibitory activity was shown by both COS and chitosan against food-borne pathogenic, spoilage bacterial and fungal strains during antimicrobial assays. Higher inhibitory effect or antimicrobial effect have been observed against *Bacillus cereus* and *Rhizopus* sp. in bread upon substitution of flour by COS (1/100 g total weight basis) as compared to use of chitosan; along with delay in the fruity odor generally present in bread (Rakkhumkaew and Pengsuk 2018). A study was conducted to determine the antimicrobial activity of COS in compression of broad-spectrum antibiotic flomox and kluacid. Four groups of COS (1, 2, 3 and 4) were fractionated

on the basis of their molecular weights by ultrafiltration. MIC was determined against *B. cereus*, *P. aeruginosa* and *C. albicans*. The inhibitory activities of COS 1 and 3 were highly effective like flomox and kluacid against *B. Cereus*. COS 4 (0.11 mg/ml concentration, less than 1.0 KDa) completely inhibited the precise growth rate of *C. albicans* and was stronger than kluacid (0.42 mg/ml). COS 3 (1–10 KDa) had 1.67 mg/ml MIC against *P. aeruginosa*. These outcomes suggest that COS can play a key role in antimicrobial activity (El-Sayed et al. 2019).

The squid processing industry generally discards squid (*Loligo formosana*) pen that is a great source of  $\beta$ -chitin. This  $\beta$ -chitin can be easily deacetylated to chitosan due to its higher reactivity toward various solvents and loose structure, further generating COS upon hydrolysis using non-specific enzymes (Singh et al. 2019a). In presence of reactive groups like amino, hydroxyl groups and smaller size, COS has more antioxidant and antimicrobial activity than chitosan (Lodhi et al. 2014). In another study, COS–EGCG (epigallocatechin-3-gallate) conjugates were prepared to determine the antimicrobial potential against *E. coli* and *Listeria monocytogenes*. The MIC values for *L. monocytogenes* (Gram-positive) were observed to be higher for both COS and C1-E0.5-conjugate as compared to *E. coli* due to its thick cell wall, therefore more tolerant against antimicrobial agents (Singh et al. 2020). The antibacterial potential of substances can be determined by MBC/MIC ratio (Minimum Bactericidal Concentration/Minimum Inhibitory Concentration) (Singh et al. 2019b). Antimicrobial agent with MBC/MIC ratio  $\leq 2$  is bacteriostatic and MBC/MIC ratio  $> 2$  is bactericidal. For COS, the MBC/MIC ratios against *L. monocytogenes* and *E. coli* were 1 and 2, respectively. For C1-E0.5-conjugate, MBC/MIC ratios were 1 and 5 toward *L. monocytogenes* and *E. coli*, respectively, suggesting COS activity as a bactericidal agent for both bacteria, whereas C1-E0.5-conjugate as bactericidal and bacteriostatic agent for *E. coil* and *L. monocytogenes*, respectively (Singh et al. 2020). COSs antimicrobial activity against several bacteria and yeasts such as *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *S. epidermidis*, *E. faecalis* and *C. albicans* was determined using microdilution method in Mueller Hinton broth. A higher antimicrobial effect upon Gram-positive bacteria and *Candida* yeast was exhibited by COS. The MIC for *S. epidermidis* and *C. albicans* were less than 0.25 and 0.5 mg/ml, respectively (Silva et al. 2021). Therefore, the broader biological activity of COSs represents them as active agents for use in various industrial sectors.

### 3 Future Prospects

Presently, there is a need for novel compounds in the pharmaceutical industry showing potent biological activities. Antimicrobial resistance has created an emergency of novel antibiotics; therefore, COS-mediated derivatives can be an alternate of novel antimicrobial agents against various microbial-mediated diseases. The research on biological activities of COS will provide a new emerging area for emerging biotechnological industries. Moreover, entire knowledge regarding the molecular study of biological activities for COS is not available. Therefore, there is a great

need for prospective research centered on the molecular functioning so that complete biochemical functioning of COS could be known.

## 4 Conclusion

COS has a vast range of biological properties and significant potential for numerous industrial applications. Upon citing the previous literature on the antimicrobial activity of chitooligosaccharide, its utilization as a potential antimicrobial agent against several pathogens like bacteria, yeast and fungi have been summarized. There is a great need for well characterization of COS for utilizing it as antimicrobial agent in food, medical and agriculture sectors.

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