



Microbial chitinases: properties, current state and biotechnological applications

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Abstract

Chitinases are a group of hydrolytic enzymes that catalyze chitin, and are synthesized by a wide variety of organisms. In nature, microbial chitinases are primarily responsible for chitin decomposition. Several chitinases have been reported and characterized, and they are garnering increasing attention for their uses in a wide range of applications. In the food industry, the direct fermentation of seafood, such as crab and shrimp shells, using chitinolytic microorganisms has contributed to increased nutritional benefits through the enhancement of chitin degradation into chitooligosaccharides. These compounds have been demonstrated to improve human health through their antitumor, antimicrobial, immunomodulatory, antioxidant, and anti-inflammatory properties. Moreover, chitinase and chitinous materials are used in the food industry for other purposes, such as the production of single-cell proteins, chitooligosaccharides, *N*-acetyl D-glucosamines, biocontrol, functional foods, and various medicines. The functional properties and hydrolyzed products of chitinase, however, depend upon its source and physicochemical characteristics. The present review strives to clarify these perspectives and critically discusses the advances and limitations of microbial chitinase in the further production of functional foods.

Keywords Chitooligosaccharides · Chitinase · Food · Microbial

Introduction

Chitin is the second most abundant carbohydrate polymer in the world. It is a biopolymer made up of *N*-acetyl D-glucosamines that are connected via β -1,4-glycosidic linkages (Muzzarelli 2013). Chitin exists naturally as the structural polysaccharide within the exoskeleton and the shell of crustaceans and within fungal cell walls. Biomass that contains high proportions of shellfish, such as shrimp, crab, and krill, has been used for a broad range of applications in agriculture, food industries, and medicine (Rinaudo 2006). Chitin and its derivatives are also used in wastewater treatment (Bhatnagar and Sillanpää 2009), drug delivery (Ahsan et al. 2018; Dev et al. 2010), as wound healing accelerators, and as dietary fiber (Cremar et al. 2018). Moreover, chitin may be useful in regenerative medicine (Dutta 2016). Chitin is, however, challenging in regard to its isolation, solubilization,

and manipulation to generate materials with desired forms and properties.

Chitinases (EC 3.2.1.14), enzymes that hydrolyze chitin, are synthesized by various microbes, including viruses, bacteria, and fungi, and by insects, higher plants, and animals (Hamid et al. 2013). In microorganisms, chitinases are produced to allow these organisms to parasitize other fungi or nematode eggs, and the degradation of chitin results in the formation of derivative nutrient sources such as nitrogen and carbon (Itoh and Kimoto 2019). Chitinases can be classified according to their catalytic mechanism into endochitinases (EC 3.2.1.14) and exochitinases. Endochitinases are characterized by their ability to randomly hydrolyze chitin along the internal chain to generate soluble oligomers of *N*-acetylglucosamine (GlcNAc). Exochitinases have been further classified into two subcategories that include chitobiosidase (EC 3.2.1.29), which releases diacetylchitobiose at the non-reducing end of chitin microfibrils, and whole *N*-acetylglucosaminidase (EC 3.2.1.30), which cleaves *N*-acetylchitooligosaccharides to generate GlcNAc monomers (Hamid et al. 2013).

Considering the importance of bioconversion of the chitinous biomass and the role of chitinases in the development

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of novel value-added products, the present review primarily highlights the diversity of chitinases based on their microbial origin, mode of action, and applications within the food industry.

Hydrolysis reaction and mechanism of action of chitinase

The classification and nomenclature of chitinase enzymes are both discussed in this section (Fig. 1). Chitinases are glycosyl hydrolase enzymes that can break down the chitin polymer by hydrolyzing the *N*-acetyl- β -D-glucosaminide (1 $\rightarrow$ 4)- β -linkages randomly into oligo- and monomeric components. The complete hydrolysis of chitin into monomers of β -*N*-acetyl-D-glucosamine requires the synergistic action of at least three enzymes, including endochitinases (EC 3.2.1.14) and the two subgroups of exochitinases (chitobiosidases [EC 3.2.1.29] and β -*N*-acetyl glucosaminidases [EC 3.2.1.30]). Endochitinases randomly split chitin at internal sites along the polymer, generating soluble, low molecular mass GlcNAc oligomers, while chitobiosidases catalyze the non-reducing end of the chitin chain or the oligomeric products of endochitinases to yield (GlcNAc)₂ as the major product. Further, *N*-acetylglucosaminidases split the β -(1,4)-glycosidic bond of (GlcNAc)₂ to generate monomers of GlcNAc (Berini et al. 2018). Certain bacteria can also produce chitosanase (EC 3.2.1.132) to deacetylate chitin to chitosan, ultimately resulting in its conversion to

glucosamine residues (Cardozo et al. 2017; Elieh-Ali-Komi and Hamblin 2016). Although numerous chitinases have been reported from a broad range of bacterial species, only a few of them are catalysts that possess broad specificity among the endochitinases, chitobiosidases, and β -*N*-acetyl glucosaminidases. These enzymes can withstand extreme conditions and are more versatile, eco-friendly, efficient, and economical for the production of chitin and chitoooligosaccharides. Successful use of these chitinases for commercial purposes will be possible only through an understanding of the catalytic mechanism of the broad specificity chitinases, which is still undiscovered. Currently, the first three-dimensional structure of a chitinase synthesized by *Bacillus thuringiensis* has been reported (Juárez-Hernández et al. 2019). This provides a foundation for future studies of chitinases in regard to their structures, catalytic sites, and interactions with inducers and inhibitors, and these studies will be aided by harnessing the advantages of bioinformatics, genome sequencing, and protein engineering.

Microbial chitinase

The chitinase enzyme is widely synthesized by living organisms within all the kingdoms. As documented extensively, bacteria and fungi appear to be the dominant chitin decomposers, and they play an essential role in the biogeochemical cycles of carbon and nitrogen in nature (Keyhani and Roseman 1999). Chitinases are also found in viruses, insects,

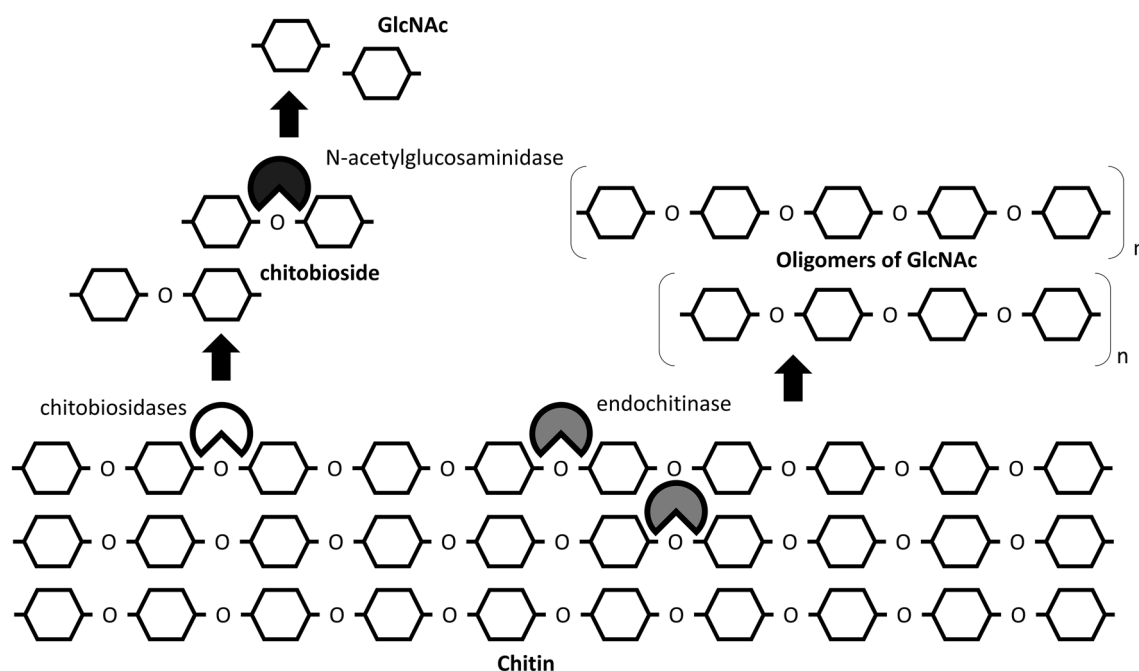


Fig. 1 Distribution of deposited chitinase proteins from different sources in UniProt database

plants, and vertebrates, and are responsible for various physiological functions such as morphogenesis, pathogenesis, parasitism, and defense (Funkhouser and Aronson 2007; Gortari and Hours 2008; Kramer and Muthukrishnan 1997). Chitinase enzymes are classified in the UniProt database into 3967 protein sequences of cellular organisms, 5 of viral origin, and 406 of metagenomes (<https://www.uniprot.org/>) (Consortium 2018). According to the database, chitinolytic bacteria accounted for 63% (2779) of the deposited proteins. The most significant bacterial groups were members of the phyla Proteobacteria and the Terrabacteria group that includes Actinobacteria, Firmicutes, Chloroflexi, Cyanobacteria, Deinococci, and Tenericutes. In addition to their central role of producing carbon and energy sources in chitin hydrolysis, chitinases also participate in antagonistic interactions (Adrangi and Faramarzi 2013; Brzezinska et al. 2014). In fungi, chitinases also play a major role in the autolytic and morphogenetic functions associated with mycoparasitism (Benhabiles et al. 2012). These fungal chitinases were identical to the 236 sequences available in UniProt database (Fig. 2). Additionally, certain characterized chitinases from microbial sources have been reported, and they are summarized in Table 1.

Chitinolytic bacteria

Bacterial chitinases are promising for use in a number of commercial applications owing to their advantages that include adaptability to a wide range of environments, short generation times, high enzyme thermostability, and ease of genetic engineering. Chitinases derived from various bacterial strains such as *Serratia marcescens* (Monreal and Reese 1969), *Bacillus* sp. (Pan et al. 2019; Tanaka and Phaff 1965), *Streptomyces* spp. (Berger and Reynold 1958), *Paenibacillus* sp. IK-5 and FPU-7 (Kusaoke et al. 2017), *Vibrio* spp. (Jahromi and Barzkar 2018), and *Salinivibrio* sp. (Le and Yang 2018) have been widely used. These bacteria have been reported to exist in various natural habitats such as soil, water, hot springs, sediment, and goldmines. Recently, *Listeria monocytogenes*, a species of pathogenic bacteria that causes the infection listeriosis, was also reported to produce chitinase (ChiA and ChiB) (Halbedel et al. 2019). These chitinases allow these bacteria to compromise immune systems, resulting in invasive listeriosis in humans.

Most bacterial chitinases are highly conserved within the GH18 family. Only few chitinases derived from actinomycetes and purple bacteria are assigned to the GH19 family

Fig. 2 Mechanism of hydrolysis of glycosidic bonds in chitin

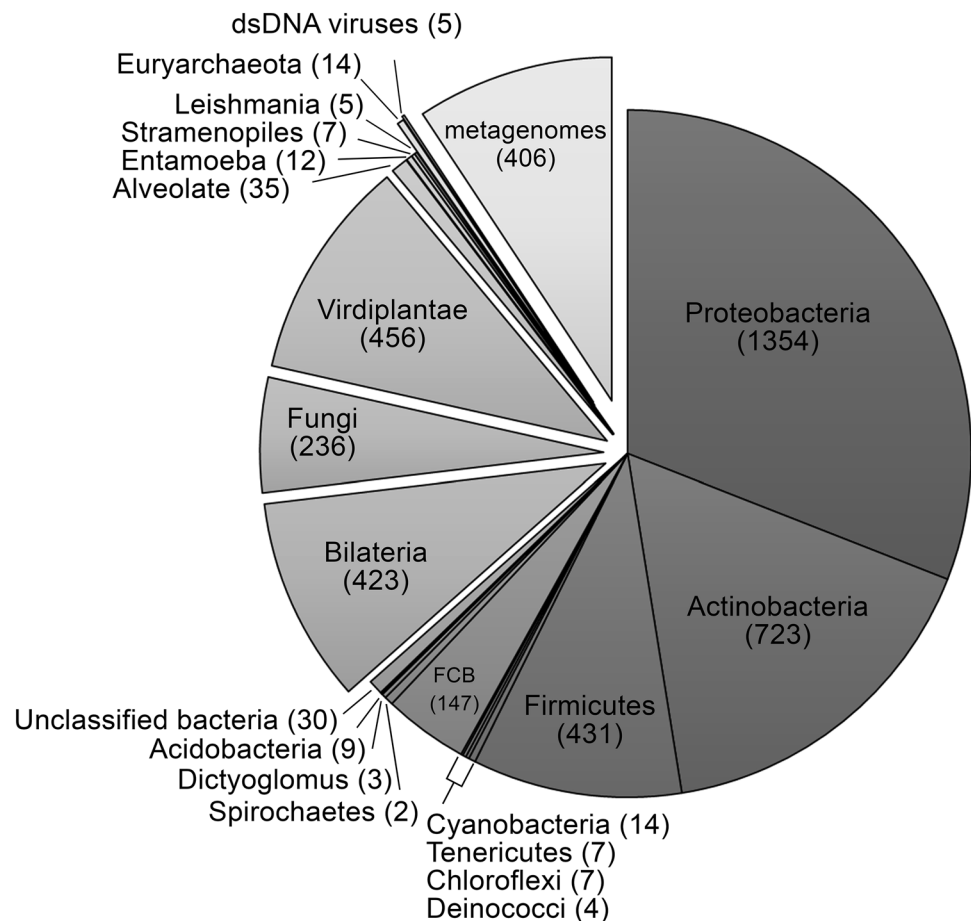


Table 1 Characteristics of chitinase from different microbial sources

No.	Organism	Gene/protein name	Glycoside hydrolase family (GH)	References
<i>Bacteria</i>				
1	<i>Serratia marcescens</i> BJL200	A, B, C1, and C2, D	GH18	Monreal and Reese (1969), Tuveng et al. (2017) and Vaaje-Kolstad et al. (2013)
2	<i>S. marcescens</i> GPS5	D	GH18	Madhuprakash et al. (2019) and Vaikuntapu et al. (2016)
3	<i>S. griseus</i> HUT6037	C	GH19	Berger and Reynold (1958) and Watanabe et al. (1999)
4	<i>Streptomyces plicatus</i>	Chitinase-63	GH19	Robbins et al. (1988)
5	<i>S. albolongus</i> ATCC 27414	A4	GH18	Gao et al. (2018)
6	<i>S. thermodiastaticus</i> HF 3–3	Chi1	GH18	Take et al. (2018)
7	<i>Streptomyces</i> sp. F-3	Chi18A, Chi18B, and Chi18C Chi19A	GH18 GH19	Sun et al. (2019)
8	<i>Bacillus circulans</i> WL-12	A1, A2, B1, B2, C and D	GH18	Tanaka and Phaff (1965) and Watanabe et al. (1992, 1993)
9	<i>B. thuringiensis</i>	A74	GH18	Juárez-Hernández et al. (2019)
10	<i>Bacillus</i> sp.	Chisb	GH18	Lee et al. (2007) and Pan et al. (2019)
11	<i>Vibrio alginolyticus</i> H-8	B A, C1, C3, D, and E	GH18 –	Ohishi et al. (2000)
12	<i>Paenibacillus</i> sp. FPU-7	A, B, C, D, E, F, and W	GH18	Itoh et al. (2013)
13	<i>Paenibacillus</i> sp. IK-5	A and B D	GH18 GH19	Kusaoke et al. (2017)
14	<i>Salinivibrio</i> sp.	C	GH18	Le and Yang (2018)
15	<i>Chitinolyticbacter meiyuanensis</i> SYBC-H1	Chi1	GH18	Zhang et al. (2018)
16	<i>Listeria monocytogenes</i>	B	GH18	Halbedel et al. (2019)
17	<i>Chromobacterium violaceum</i> ATCC 12472	Chi47	GH18	Sousa et al. (2019)
<i>Fungi</i>				
18	<i>Saccharomyces cerevisiae</i>	Cts1 Cts2	GH18 –	Hurtado-Guerrero and van Aalten (2007) and Kuranda and Robbins (1991) Giaever et al. (2002)
19	<i>Beauveria bassiana</i>	Chit1	GH18	Pinnamaneni et al. (2010)
20	<i>Metarhizium anisopliae</i>	A, B, C, D, and E	GH18	Junges et al. (2014)
21	<i>Ustilago maydis</i>	Cts1, Cts2, and Cts3	GH18	Langner et al. (2015)
22	<i>Coprinopsis cinerea</i>	E1	GH18	Zhou et al. (2018)
23	<i>Trichoderma harzianum</i>	Chit46	GH18	Deng et al. (2019)
24	<i>Xenorhabdus nematophila</i> HB310	Chi60 and Chi70	GH18	Liu et al. (2019)

(Terrapon et al. 2017). Although both GH18 and GH19 endochitinases catalyze the degradation of chitin, they share no sequence similarity, three-dimensional protein structures, and catalytic mechanisms, indicating independent evolutionary origins (Adrangi and Faramarzi 2013). The GH18 family chitinases possess a catalytic site that contains a TIM barrel (Huang et al. 2011). A glutamate residue plays an important role in the binding of substrates to the active site of the GH18 catalytic domain, and this can result in unpredictable substrate binding and subsequent catalytic products. In general, most of the bacterial chitinases were

isolated using solid media containing chitin or its derivatives (soluble, swollen, or colloidal chitin). Chitinases have been characterized by homologous or heterologous protein expression in *Escherichia coli* after serial purification processes (Dahiya et al. 2006; Sahai and Manocha 1993). The earliest isolated and the most important species for the degradation of chitin is *S. marcescens*, which is also considered to be a model bacterium for the study of enzymatic activities (Vaaje-Kolstad et al. 2013). *Serratia marcescens* produces five different chitinolytic enzymes. The *S. marcescens* chitinases ChiA, ChiB, ChiC1, ChiC2, and ChiD are

members of the GH18 family, and the lytic polysaccharide monooxygenase chitin-binding protein CBP21 belongs to the auxiliary activity (AA) family 10. Both ChiA and ChiB produce disaccharides by moving along the chitin chains in opposite directions, while ChiC works as an endo-acting non-processive chitinase and hydrolyzes the chitin polymers randomly (Tuveng et al. 2017; Vaikuntapu et al. 2016). Although the thermodynamic signatures for binding of (GlcNAc)₆ and the inhibitor allosamidin to SpChiD have been identified (Madhuprakash et al. 2019), knowledge regarding the role of ChiD in chitin degradation remains limited. In *Bacillus circulans*, at least ten chitinases have been reported and classified as ChiA, ChiC, and ChiD (Watanabe et al. 1992, 1993). Chitinase D from *B. circulans* is cleaved at both the N-terminal and C-terminal domains to generate chitinase B1 and B2 (Gao et al. 2018; Take et al. 2018; Watanabe et al. 1992, 1999). The *Streptomyces* spp. contain the highest number of chitinases reported (Robbins et al. 1988; Yandigeri et al. 2015). These chitinases also belong to the GH18 family (11 chitinases) and the GH19 family (2 chitinases). In *Paenibacillus* sp. IK-5, two GH18 chitinases (ChiA and B) and one GH19 chitinase (ChiD) have been cloned and sequenced (Itoh and Kimoto 2019). In *Vibrio* spp. (the pathogenic marine bacteria), there is rapid cycling of chitin through the two-component signaling system of the binding protein for chitin oligosaccharides (CBP) periplasmic domain of the membrane protein (ChiS) (Meibom et al. 2004). In a study performed by Sousa et al. (2019), antagonistic *Chromobacterium violaceum* exhibited high chitinase activity at elevated temperatures. This chitinase retains greater than 50% hydrolytic activity after treatment at 100 °C for 1 h. Moreover, the heterologous chitinase in *Streptomyces* sp. F-3 could withstand temperatures of up to 70 °C (Sun et al. 2019). Thermo-stable chitinase can be used for the industrial-scale production of chitooligosaccharides.

Chitinolytic fungi

Although the chitinase genes are distributed widely throughout fungal genomes, most of the chitinases are members of the GH18 family, with the exception of chitinase from *Nosema bombycis* (Han et al. 2016). Chitinase derived from *N. bombycis* was the first GH19 chitinase to be reported in fungi. Based on sequence similarity and domain organization, fungal GH18 chitinases were classified into A, B, C, and D subfamilies (Junges et al. 2014). These fungal GH18 chitinase subfamilies do not, however, correspond to the eponymous subfamilies among the bacterial GH18 chitinases, and they instead exhibit high amino acid homology to class III plant chitinases (Duo-Chuan 2006). The fungal chitinase genes are composed of five domains, including the catalytic domain, chitin-binding domain, serine/

threonine-rich domain, N-terminal signal peptide region, and C-terminal extension region (Hamid et al. 2013).

Similar to those of bacteria, the fungal chitinases not only play an important role in degradation but also in morphogenesis, cell division, autolysis, and acquisition of chitin (Langner and Göhre 2016; Rathore and Gupta 2015). Filamentous fungi possess more expanded chitinase gene families (seven groups) than do yeast (Stoykov et al. 2015); however, the functions of individual chitinases remain unknown because of the difficulty in knocking down multiple genes to characterize phenotypic changes. For example, *Ustilago maydis* produces three chitinases (UmCts1, UmCts2, and UmCts3). The chitinases UmCts1 and UmCts2 are essential for cell separation, while UmCts3 is thought to compete with other microbiomes (Langner et al. 2015). In *Saccharomyces cerevisiae*, the disruption of ScCts1 resulted in the formation of multicellular aggregates because of defective cell separation. The ScCts1p plays an important role in coordinating cell wall degrading activities (Hurtado-Guerrero and van Aalten 2007; Kuranda and Robbins 1991), while ScCts2 is involved in ascospore formation (Giaever et al. 2002). The production of chitinase has been reported in mycoparasitic and entomopathogenic fungi (Deng et al. 2019). According to Deng et al. (2019), Chit46 chitinase derived from *Trichoderma harzianum* inhibits the growth of the phytopathogenic fungus *Botrytis cinerea*. This chitinase hydrolyzes chitin to GlcNAc₂ with an efficiency of up to 94.8%.

Fungal chitinases have been isolated and characterized for use in possible applications that include the production of biocontrol agents, chitin hydrolysis, and the production of virulent forms. Thermophilic fungi exhibit a significantly high activity and thermal stability, and can endure harsh conditions. Furthermore, engineered chitinases exhibited increased virulence compared to that of the native enzyme in *Trichoderma harzianum* and *Beauveria bassiana* (Mythili et al. 2018; Selenius et al. 2018). This approach provides crucial information regarding the production of bioactive chitooligosaccharides and the development of high-efficiency agents to control plant diseases and other harmful fungi. Indeed, owing to limitations in the number of identified fungal chitinase sequences, it is clear that our knowledge of the molecular pathways underlying the functions of fungal chitinases is lacking (Fig. 2).

Benefits and production of chitooligosaccharides

Chitooligosaccharides (COSs), low-molecular-weight degraded products of chitosan or chitin, are water soluble bioactive molecules that can be easily absorbed by the human intestine (Liang et al. 2018). Although COSs and its commercial products have been approved by international

regulatory organizations, the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) have established safe daily intake levels for certain COS products such as chewing gums, bandages, and drug delivery systems (Liaqat and Eltem 2018). The production of COSs from natural sources is substantial in Asian countries, as these countries are the major exporters of crustaceans. A larger amount of attention has been paid to shellfish waste in recent years owing to the annual production of over 1.44 million metric tons of waste (dry weight) worldwide (Rødde et al. 2008). Rapid growth within the seafood processing industry has raised serious concerns regarding disposal issues because of the low biodegradation rate of this waste. Large amounts of shellfish waste have been dumped into the oceans, resulting in pollution of the coastal areas. Shellfish, however, can be a valuable resource when managed reasonably, and ultimately it can be used to reduce environmental problems and can add great economic value to the production of bioactive compounds. To date, only small amounts of waste chitin have been recycled, despite the fact that shellfish waste possesses a 20–30% chitin content (Kumari et al. 2015). In the last decade, several studies have demonstrated various biological activities of COS. Chitooligosaccharides have been proposed as potential prebiotics, as they are degraded by different bacterial strains within the human colon. Lee et al. (2002) demonstrated that *Bifidobacterium* and *Lactobacillus* probiotic strains metabolized different types of fructooligosaccharides (FOS) that possessed a degree of polymerization of 2–8 (DP 2–8). These effects, however, are dependent upon the molecular weight and the degree of acetylation of COS (Selenius et al. 2018). Reportedly, COS reduces the growth of some food pathogenic bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Shigella dysenteriae*, and *Salmonella typhimurium* (Benhabiles et al. 2012; Fernandes et al. 2008). Based on this, COS has been proposed for use as a food biopreservative, and COS prebiotic properties can allow it to serve as a functional food. Recently, COS has been used in some functional foods such as milk, apple juice, yoghurt, and tofu (Fernandes et al. 2008; Patel and Goyal 2011). In low COS concentrations (2 g/L), *L. acidophilus* exerts a larger inhibition zone against *E. coli* and *Staphylococcus aureus* than that exerted by *L. acidophilus* alone (Miksusanti et al. 2016).

Chitooligosaccharides have received a great deal of attention for use in novel anticancer therapeutic strategies. Suzuki et al. (1986) revealed that hexa-*N*-acetylchitohexaose and chitohexaose inhibit the growth of S180 and MM46 solid tumors in mice. Furthermore, studies on the underlying mechanism by Park et al. (2011) have demonstrated that COSs promote innate immune defense and suppress tumor cells by activating cytokine secretion and cytotoxic T lymphocyte proliferation. Moreover, COS suppresses colorectal tumor progression by AMPK activation,

and through NF- κ B and mTOR signaling inhibition (Mataveewong et al. 2016). Zou et al. (2018) discovered that in vitro, COS (DP 2–6) significantly inhibited cell proliferation and caused cell cycle arrest at S phase, and it also reduced the tumor weight by up to 58.6% in vivo. They also found that COS influences the regulation of PI3K/Akt, p38 MAPK, and p53 pathways to inhibit hepatoma progression (Zou et al. 2019).

Chitooligosaccharides are well-known anti-inflammatory agents. They suppress inflammation within the colonic mucosa by influencing various complex pathways. In general, COSs are involved in decreasing the lipopolysaccharide (LPS)-induced secretion of nitric oxide (NO), modulation of TNF- α pathway, depletion of glutathione (GSH) and catalase (CAT) levels, increasing malondialdehyde (MDA) levels, and activating nuclear factor-kappa B, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase. The different efficacies of the administered COSs, however, may result from the differences in their molecular weight, degree of polymerization, *N*-acetylation, arrangement of acetyl groups based on binding affinity, and conformation for COS-protein interaction (Aam et al. 2010; Bekale et al. 2015). In the study conducted by Chang et al. (2019), small chitosan (7.1) and COS activated NF- κ B and enhanced iNOS expression by binding to CD14, TLR4, and CR3 receptors to activate JNK signaling proteins that are not found in higher molecular weight chitosan. Additionally, the presence of higher number of deacetylated reducing ends could also contribute to pro-inflammatory effects. Therefore, identification of the most active molecules is required.

Numerous studies have reported coat-based vaccine properties of COS resulting from its biological activities, higher water solubility, and lower molecular weight compared to those of chitosan. Thanou et al. (2002) assessed efficient gene delivery of COS in COS-1 and Caco-2 cell lines. They observed that COS possesses no dose-dependent cytotoxicity, and COS could condense DNA and form complexes in both the cell lines. Collectively, these results indicate that COS possesses potent transfection efficiency as a novel gene-based vaccine. It was reported that the complex of COS (3 kDa), mPEI, and rAd5 elicited a stronger antigen-specific CTL response by significantly activating CD8⁺ T cells positive for IFN- γ , and CD4⁺ T cells positive for IL-4 (Jiang et al. 2014). Moreover, in a recent study, COS was used to coat the recombinant *Listeria ivanovii* (LI)-based vaccine candidate strain LI-Rv0129c expressing *Mycobacterium tuberculosis* (*Mtb*) antigen Ag85C. The COS-coated LI-Rv0129c complex improved the ratio of particles phagocytosed by RAW 264.7 macrophages and elicited CD4⁺ IFN- γ , CD4⁺ TNF- α , or CD8⁺ IFN- γ secretion.

Application of chitinase in the food industry

Chitinases have shown immense potential for a wide range of applications involving food and medicine. Applications using chitinase-based processing are economic, eco-friendly, and non-hazardous, and these applications are further discussed in the following subsections.

Production of chitooligosaccharides and GlcNAc

Several techniques have been used for the preparation of COS and GlcNAc, and these include chemical degradation, chemical synthesis, physical degradation, and enzymatic methods. Among these techniques, enzymatic degradation has attracted increasing attention owing to its advantages that include strong biological activity, simple processing, mild conditions, and ecofriendly (Aam et al. 2010). The use of this complex enzymatic method for the synergistic degradation of chitin and for producing pure enzymes, however, remains expensive because of a low conversion rate (Jung and Park 2014).

Chitopentaose and chitotriose are the main products (up to 60%) generated from colloidal chitin (prepared from crab shell flake chitin) using chitinase derived from *Vibrio alginolyticus* TK-22 (Murao et al. 1992). In another study by Samain et al. (1997) up to 2.5 g/L of a mixture of penta-*N*-acetyl-chitopentaose and tetra-*N*-acetyl-chitopentaose was produced by recombinant *E. coli*-expressing nodC or nodBC expressed by *A. caulinodans*. Our study found that chitinase derived from *Salinivibrio* BAO-1801 can act as a suitable biocatalyst for chitobiose (Le and Yang 2018). The use of a combination of different chitinolytic enzymes would be necessary to obtain the desired chain length of the oligomer. For example, Suresh and Kumar (2012) used *Penicillium monoverticillium* CFR 2, *Aspergillus flavus* CFR 10, and *Fusarium oxysporum* CFR 8 as chitinolytic enzymes to produce GlcNAc from α -chitin. They found that the crude endochitinases from the three strains were the most effective in generating monomeric GlcNAc (~ 80%) and a small amount of (GlcNAc)₄ (~ 20%). Zhou et al. (2018) compared the COS production by exochitinase ChiE1, *Coprinopsis cinerea*, and endochitinase Chi-III alone or in combination. They found that ChiE1 could not degrade chitobiose; however, the yield of reducing sugars was increased by 120%, suggesting that chitinase conversion of chitin-to-chitin oligosaccharides typically requires both catalytic activities in a synergistic manner.

Based on the catalytic mechanism, multi-functional chitinases that possess both exo- and endo-activities along with *N*-acetyl- β -D-glucosaminidase activity are more effective than single catalytic-domain chitinases. Tanaka et al.

(2003) found that chitinase derived from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1 possesses endo- and exo-type catalytic domains together with three chitin-binding domains on a single polypeptide. Interestingly, Zhang et al. (2018) reported that CmChi1 derived from *Chitinolyticbacter meiyuanensis* SYBC-H1 is expressed in *Escherichia coli* BL21(DE3) cells to produce GlcNAc. This enzyme exhibits exo-, endo-, and *N*-acetyl- β -D-glucosaminidase activities toward *N*-acetyl COS. The potential for total GlcNAc production was observed, with nearly 98% purity after the recovery process. These results highlight the need for new strategies for discovering novel chitinase enzymes that can exert more than one catalytic activity while possessing high efficiency.

Production of single-cell proteins

Certain microbes are gaining interest owing to their ability to produce single cell proteins (SCPs), which can be used as a protein dietary supplement and can replace costly conventional protein sources such as soymeal and fishmeal (Nasseri et al. 2011). As single cells, microorganisms could utilize inexpensive waste sources to allow for growth and increased biomass. Owing to their short generation time, ease of genetic engineering, and their ability to utilize a variety of substrates, microbes possess many advantages. Shellfish waste is a rich source of chitin, CaCO₃, and protein. Tom and Carroad (1981) reported that chitinases extracted from *Pichia kydriavzevii* and *S. marcescens* hydrolyzed chitin and generated single-cell proteins with yields of 45% protein and 8–11% nucleic acids. In another study, *Myrothecium verrucaria* and *S. cerevisiae* chitinolytic enzymes transformed chitin waste to single-cell proteins and ethanol (Kuranda and Robbins 1991; Vyas and Deshpande 1991). Patil and Jadhav (2014) used an enzymatic hydrolysis system where *Penicillium ochrochloron* MTCC 517 converted chitin to *N*-acetyl-D-glucosamine and *Yarrowia lipolytica* NCIM 3450 then further generated single-cell proteins. They found that up to 2% chitin was sufficient for achieving optimal single-cell protein yield, with a maximum yield of 65% protein and 2.9% nucleic acids from 9.4 g/L of biomass. Additionally, this yeast single-cell protein could be replaced with 50% fish meal to induce growth of the fish *Lepidocephalus thermalis* (Wadhwa and Bakshi 2016). These studies suggest that chitinases exhibit promising new properties to produce single-cell proteins for use as cheaper alternative protein sources, particularly in the context of converting marine or aquaculture waste to fishmeal.

Biocontrol of post-harvest fungal pathogens

Biological control of post-harvest diseases has also garnered interest over the past years because of the adverse effects

of chemicals on human health and the environment and to the development of fungicide-resistant strains (Chalutz and Droby 1998). Biocontrol using microorganisms has emerged as a promising alternative that possesses advantages such as feasibility and cost-effectiveness (Sharma et al. 2009). Several microorganisms exhibited promising antifungal effects against major postharvest pathogens (Castillo et al. 2016; Le and Yang 2018). Chitinolytic enzyme-producing bacteria, as potential biocontrol agents of phytopathogens, break down the cell wall structure. Chitinase treatment can increase food preservation time, likely by damaging the cell wall, preventing spore germination, and ultimately reducing the deterioration of food. The antifungal activity of chitinase has been observed in both bacteria and fungi. For example, our study demonstrated that ChiC chitinase from *Salinivibrio* (Le and Yang 2018) exhibited antifungal activity against the pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani*. In another study, Deng et al. (2019) demonstrated that chitinase from *Trichoderma harzianum* effectively inhibited the growth of the phytopathogen fungus *Botrytis cinerea* at doses ranging from 1.5 to 6.0 µg/mL. According to Liu et al. (2019), the co-expression of two chitinases (chi60 and chi70) in *Xenorhabdus nematophilus* inhibited spore germination by *Verticillium dahliae* and *Coniella diplodiella*. Based on these findings, it is clear that chitinases are valuable as a new source of antifungal agents that have potential applications within the food industry for the storage of fruits and vegetables.

Tannase recovery

Tannase is a recently commercialized enzyme that is commonly used as a preservative in the food industry to generate gallic acid and to remove unwanted hydrolyzable tannins (Belmares et al. 2004). Foods that contain high amounts of tannins are dangerous, and thus, tannase is used to reduce these tannins. Tannase produced from *Aspergillus niger*, however, is strongly bound to the mycelium, consequently resulting in reduced activity and yield (Lekha and Lonsane 1994). Barthomeuf et al. (1994) found that chitinase derived from *Streptomyces griseus* can recover 43% of the tannase yield by reverse tannase extraction.

Functional food and medicine

Chitinases can be used for a number of human healthcare applications such as the production of ophthalmic preparations with chitinases and microbiocides. Owing to the antifungal activity of chitinase, there has been an unfortunate gap in the development of a naturally occurring agent and therapy for fungal diseases (Halder et al. 2019). Antifungal drugs combined with essential amounts of chitinase have been used as a therapeutic treatment for various fungal

infections (Gutiérrez-Román et al. 2015). According to Allonsius et al. (2019), some *Lactobacillus* sp. strains, especially the taxa encompassing the *L. casei* group, can produce chitinases capable of breaking down the main polymer of the hyphal cell wall of *Candida albicans*. The synergistic effects of chitinase and lactic acid have been observed to enhance the growth inhibition of *C. albicans*, and these effects can assist in the selection of proper probiotic strains for use as potential probiotics in patients with *Candida* infections. However, the growth of marine *Pseudoalteromonas* sp. on chitin produced novel antimicrobial compounds that could lead to new bioprospecting strategies for the treatment of infectious diseases (Paulsen et al. 2019).

Additionally, crude chitinase mixtures derived from *S. marcescens* and *S. griseus* possess prodrug activities and can inhibit the growth of and destroy cancer cells (MCF-7 and B11-2) in combined immunodeficiency mice (Pan et al. 2005). Based on these findings, the use of chitinase as a clinical test reagent should be further explored, as to the best of our knowledge, very few chitinases have been reported to exhibit cytotoxicity toward mammalian cells.

Challenges and future perspective

The rapid and ecofriendly chitinase production by microorganisms shows great promise of possible applications within the food industry; however, there are still several aspects limiting chitinase use, including optimal industrial-scale studies, low-yield purification process of crude enzyme, and low enzyme activity. Additionally, production costs and other unknown mechanisms hinder their application in the food industry. Further studies are required in the near future to facilitate the improvement of chitinase activity. For example, ultrasound treatment enhanced the efficacy of chitinase enzyme by 19.17% (Hou et al. 2019). Moreover, genome editing may be safer to use and could further enhance efficacy. The heterologous expression of Chisb in *B. subtilis* WB600 increased the expression level and the specific activity of Chisb to 51.67 U/mL and 249.62 U/mg, respectively. Mutation of the catalytic alanine residue significantly reduced the hydrolase activity of *Thermococcus kodakaraensis* ChiA, reducing the formation of insoluble polymers to a 45% yield (w/w) that only contained approximately 55% of the target GlcNA10 (Alsina et al. 2019). Although numerous chitinases have been purified and characterized, the exact mechanisms and the interactions of the different chitinases remain unclear. Further mechanistic studies are urgently required. Chitinases from other kingdoms, such as Plantae and Protista, are still unknown, although these enzymes have been well characterized in fungi and bacteria. State-of-the-art second-generation sequencing technologies such as RNA interference or high-throughput surveys

of DNA–protein interactions will be particularly helpful for identifying whole family genes and understanding the evolution of chitinase across species. Transcription profiling studies have helped identify genes related to chitinase pathways and processes.

Current studies are being performed in an attempt to improve the enzyme performance by modifying genes and determining the structural and functional properties of the protein. Site-directed mutagenesis is an invaluable tool for altering, inserting, or deleting single amino acid residues, multiple residues, or even entire structural elements that directly enhance chitinase properties such as substrate conversion, product specificity, stability, and specific activities. There are no studies regarding the identification of food allergenic chitinase produced by microorganisms (Leoni et al. 2019). In particular, many GH18 chitinases in plants and insects were identified as food allergens, indicating that further efforts are still needed to increase our knowledge related to the diversity and ecology of these microorganisms. Currently, chitinous materials have begun to gain recognition within the food and medicine sectors owing to their uses for spoilage prevention, drug-delivery, and anticancer therapy. Further research is essential to increase market demand for chitinolytic enzymes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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