Microbial Chitinases — Production, Characterization, Purification and their Biotechnological and Therapeutic Applications: An Integrated Review

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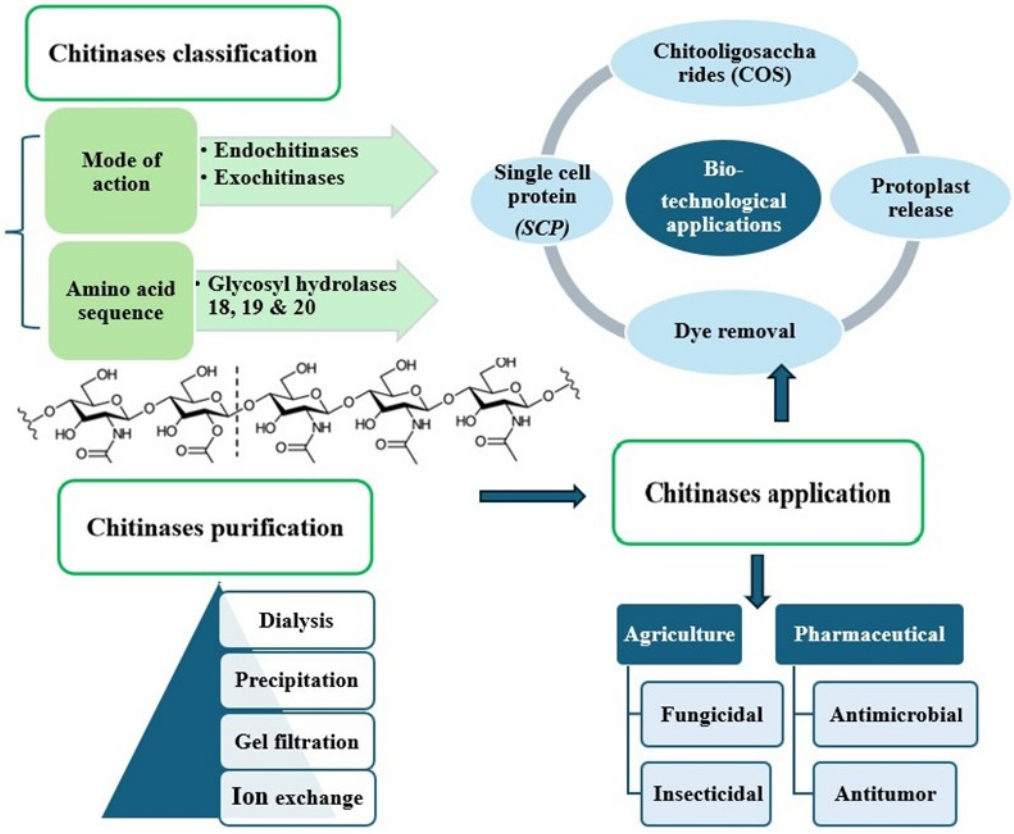
GRAPHICAL ABSTRACT



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Chitin is the second most abundant natural polysaccharide after cellulose and consists of N-acetyl-D-glucosamine units linked by β-1,4-glycosidic bonds. In nature, chitin does not accumulate due to the synergistic action of chitinolytic enzymes. Based on their catalytic domains, chitinases are classified into glycosyl hydrolase families GH18 and GH19. They are widely produced by bacteria and filamentous fungi. Different types of chitinolytic enzymes, including endochitinases, exo-acting enzymes, and N-acetylglucosaminidases, have been reported to exhibit antimicrobial and insecticidal activities, making them valuable tools for controlling phytopathogenic fungi and insect pests. Chitin degradation generates chitooligosaccharides (COS), which possess diverse biological properties such as antimicrobial, antioxidant, anti-inflammatory, and antitumor activities, contributing to improved human health. Microbial chitinases are also applied in several industrial and environmental processes, including protoplast formation, single-cell protein production, and dye removal. Advances in recombinant expression and genetic engineering have enhanced chitinase production, stability, and catalytic efficiency. Moreover, recombinant chitinases have been successfully utilized in biocontrol strategies and in developing transgenic plants with increased resistance to phytopathogens. This review highlights the broad agricultural, industrial, and biomedical applications of chitinases and their crucial role in promoting environmental sustainability and advancing bio-based industrial processes.

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Keywords: Chitinases; Microorganisms; Purification; Biological control; Chitooligosaccharides; Protoplasts; Transgenic plants.

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INTRODUCTION

Chitinases (EC 3.2.1.14) are ubiquitous endo-acting glycosyl hydrolases (GH) that catalyze the cleavage of β-1,4-glycosidic bonds within chitin, the second most abundant biopolymer. This enzymatic action produces chitooligosaccharides, which can be further hydrolyzed by N-acetyl-β-D-hexosaminidases into monomeric N-acetylglucosamine (Cohen-Kupiec and Chet 1998). These enzymes are primarily grouped into two main families, the glycosyl hydrolase family 18 (GH18) and the glycosyl hydrolase family 19 (GH19), based on their distinct active sites (Udaya Prakash *et al*. 2010).

From a research perspective, chitinases present a fascinating area of study due to their diverse catalytic mechanisms and intricate molecular architecture (Berini *et al.* 2018). Investigating their substrate specificity is paramount, as it serves as a critical avenue for elucidating the precise correlations between enzymatic function and their physiological roles across various biological systems. Furthermore, a comprehensive understanding of this specificity directly informs and optimizes strategies for the bioproduction of industrially significant compounds. Notably, these enzymes demonstrate catalytic activity across a broad spectrum of chitin polymorphs, encompassing *α*-, *β*-, and *γ*-chitin, in addition to their derivatives such as chitosan and various chitooligosaccharides (Horn *et al*. 2006).

Chitinases are primarily produced by microorganisms; although similar enzymes have been reported in plants and animals, they function in plant defense against chitin-containing pathogens (Grover, 2012) and in immune responses and inflammation in animals (Garth *et al*. 2018). Microbial chitinases are increasingly favored due to their superior attributes compared to chitinases from other sources. Their production is characterized by significantly higher yields and reduced operational costs, which can be primarily attributed to their capacity to efficiently utilize abundant and inexpensive raw materials, such as chitinous shellfish waste (Karthik *et al*. 2014), insect exoskeletons (Merzendorfer and Zimoch 2003), and fungal cell walls (Langner and Göhre 2016). Furthermore, these raw materials can be pre-processed into more bioavailable forms, such as colloidal chitin, thereby optimizing enzyme production efficiency (Deng *et al*. 2019). A critical advantage lies in the inherent stability of microbial chitinases across diverse environmental conditions, coupled with their amenability to genetic engineering. This allows for targeted modifications to enhance catalytic activity or tailor specific enzymatic properties, thereby expanding their biotechnological utility (Stoykov *et al*. 2015).

Chitinases are broadly distributed across a multitude of organisms, where they fulfill critical physiological and ecological functions (Jahromi and Barzkar 2018). In bacterial systems, these enzymes are integral to nutrient acquisition, parasitic interactions, and the essential recycling of chitin (Jahromi and Barzkar 2018). Fungi leverage chitinases for fundamental processes such as spore germination, hyphal development, morphogenesis, and nutrient assimilation (Thakur *et al*. 2023). In plants, chitinases are expressed during growth as pathogenesis-related proteins, conferring protection against chitin-containing pathogens (Ali *et al*. 2020). Human physiology includes chitinases in serum, leukocytes, and gastric secretions, contributing to innate defense mechanisms against chitinous threats (Abdelraouf *et al*. 2024). Crustaceans depend on chitinases for vital processes, including molting, growth, reproduction, and defense against pathogens (Zhang *et al*. 2014).

Numerous bacterial genera, including *Aeromonas*, *Bacillus*, *Streptomyces*, *Paenibacillus*, *Serratia*, and *Pseudomonas*, have been identified as prolific chitinase

producers (Itoh and Kimoto 2019). Likewise, several filamentous fungi, such as *Aspergillus*, *Conidiobolus*, *Beauveria*, *Mucor*, *Neurospora*, *Trichoderma*, and *Penicillium*, are known to synthesize diverse chitinases (Thakur *et al*. 2023; Gupta *et al*. 2025). Among the available approaches, submerged fermentation remains the most widely employed method for microbial chitinase production (Stoykov *et al*. 2015).

Chitinases, owing to their capacity to hydrolyze chitin found in insect exoskeletons, fungal cell walls, and various other chitin-containing structures, have gained substantial biotechnological importance (Avupati *et al*. 2017). Their applications are multifaceted, encompassing roles as antimicrobial and insecticidal agents in the biocontrol of phytopathogens (Chatterton and Punja 2009). Furthermore, chitinases are central to the bioconversion of chitin into pharmacologically valuable products, such as N-acetylmuramic acid and chitooligosaccharides (Liang *et al*. 2018). These products exhibit diverse bioactivities and have been explored as antimicrobial agents, modulators of host defense mechanisms, drug delivery vehicles, cholesterol-lowering agents, and food preservatives (Rameshthangam *et al*. 2018). In addition to these functionalities, emerging evidence highlights the potential of chitinases as diagnostic biomarkers for a range of pathological conditions, including inflammatory and autoimmune disorders, asthma, oral diseases, and both acute and chronic inflammatory states (Castañeda-Ramírez *et al*. 2013). Furthermore, chitinases have been extensively investigated for their utility in fungal protoplast generation (Hassan 2014) and in the development of transgenic plants with improved resistance against phytopathogens and insect pests (Kumar *et al*. 2018).

This review aims to provide a comprehensive and integrated perspective on microbial chitinases. It encompasses their classification, mechanisms of action, microbial producers, and the various strategies employed to enhance their production, stability, purification, recombinant expression, and genetic engineering. Additionally, this review presents an inclusive overview of their therapeutic, agricultural, and industrial applications, with a particular emphasis on recent progress and emerging innovations reported in the literature.

Chitin Production and Characterization

Chitin is structurally similar to cellulose, as both are polysaccharides composed of β-(1→4)-linked glycosidic bonds; however, chitin differs in that the hydroxyl group at the C2 position of the glucose monomer is replaced by an N-acetyl group (N-acetylglucosamine), which enhances its stability, rigidity, and mechanical strength compared to cellulose (Kobayashi *et al*. 2023). Chitin exists in three main crystalline forms: *α*-, *β*-, and *γ*-chitin, which differ in molecular organization, intermolecular interactions, and physicochemical properties (Kaya *et al*.,2017). *α*-Chitin, with antiparallel polysaccharide chains, forms extensive intra- and intermolecular hydrogen bonds, resulting in a highly ordered, densely packed structure that is chemically inert and poorly soluble; it is predominantly found in fungal cell walls, insect exoskeletons, and crustacean shells (Av *et al*. 2004; Langner and Göhre 2016; Merzendorfer and Zimoch 2003; Casadidio *et al*. 2019). In contrast, *β*-chitin has parallel chains with weaker intermolecular forces, producing a more loosely packed and chemically reactive structure, mainly occurring in diatoms and cephalopod skeletal structures (Gardner and Blackwell 1975). *γ*-Chitin exhibits a mixed chain arrangement, resulting in intermediate structural order, solubility, and reactivity, and is found in some beetles and cephalopods (Kaya *et al*. 2017).

Chitin's robust molecular structure makes it highly persistent in the environment, leading to large amounts of chitin-containing waste from shrimp, crab, and other seafood

processing (Zhou *et al*. 2018). Over 60% of this waste is improperly managed, contributing to pollution due to chitin's resistance to natural degradation (Yadav *et al*. 2021). To address this problem, researchers have developed various extraction methods from chitin-containing residues, which are broadly categorized into chemical and biological approaches

Chemical extraction techniques, applied at both laboratory and industrial scales, typically necessitate the application of potent acids and bases (El Knidri *et al.* 2018). Chemical extraction of chitin typically involves three main steps: deproteinization, demineralization, and decolorization. Deproteinization is performed by treating shells with alkaline solutions, while demineralization (elimination of calcium carbonate) is carried out using acidic conditions, most commonly hydrochloric acid at elevated temperatures (Younes and Rinaudo 2015). Decolorization is achieved with organic solvents such as acetone, ethanol, methanol, and chloroform, or with inorganic agents such as sodium hypochlorite and hydrogen peroxide (Younes and Rinaudo 2015). The chemical approach, while effective, is risky and expensive, underscoring the demand for safer chitin extraction methods (El Knidri *et al.* 2018).

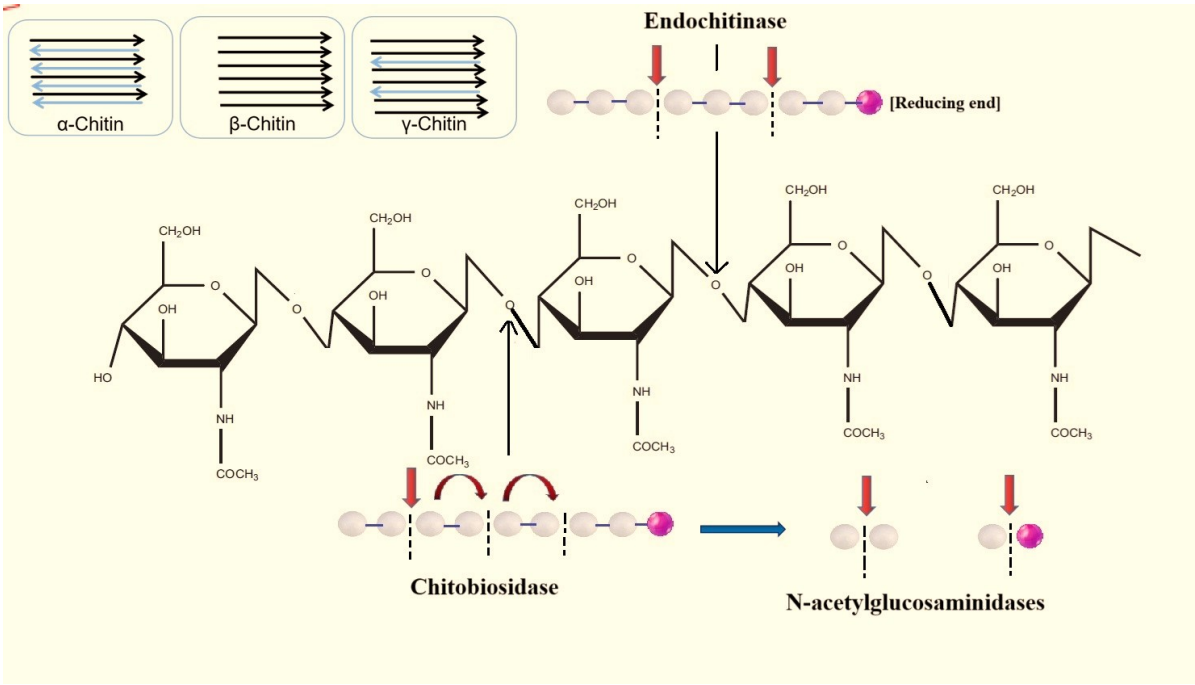

Fig. 1. Schematic representation of chitin structure and its enzymatic degradation

The biological extraction of chitin primarily involves two steps: deproteinization and demineralization. Deproteinization can be achieved either enzymatically or *via* microbial fermentation. Enzymatic methods employ proteases such as alcalase and trypsin, whereas fermentation utilizes protease-producing microorganisms such as *Aspergillus* and *Bacillus*, typically under controlled conditions. Demineralization is performed through lactic acid fermentation by *Lactobacillus*, which converts calcium carbonate into calcium lactate. The resulting chitin is then thoroughly washed, dried at low temperatures, and milled to preserve its structural integrity. This biological approach offers several advantages, including cost-effectiveness, environmental friendliness, and the production of chitin with desirable physicochemical properties (Karthik *et al*. 2014).

Mechanisms and Classification of Chitinases

Chitinolytic enzymes can be classified based on their amino acid sequences and mechanistic pathways. Broadly, they are divided into two main types according to their mode of action: endo-chitinases and exo-chitinases (Fig. 1). Endo-chitinases (EC 3.2.1.14) randomly hydrolyze chitin, producing soluble low-molecular-weight oligomers of N-acetylglucosamine (GlcNAc), such as chitotetraose, chitotriose, and di-acetylchitobiose, as well as longer oligosaccharides with a degree of polymerization greater than four (DP > 4). Exo-chitinases are further classified into chitobiosidases (EC 3.2.1.29), which release di-acetylchitobiose sequentially from the non-reducing end of the chitin chain, and 1-4-β-N-acetylglucosaminidases (EC 3.2.1.30), which hydrolyze oligomeric products into GlcNAc monomers (Cohen-Kupiec and Chet 1998). In this context, chitinases in Family GH 18 exhibit critical dual functionality encompassing both hydrolytic (bond cleavage) and transglycosylation (bond creation/synthesis) activities. While hydrolysis is the primary catabolic function, degrading chitin into smaller oligomers, transglycosylation serves as the synthetic function, creating new glycosidic bonds to synthesize longer oligosaccharides. Consequently, the equilibrium between cleavage and synthesis is critically dependent on environmental factors, notably water activity and substrate concentration (Madhuprakash *et al*. 2013).

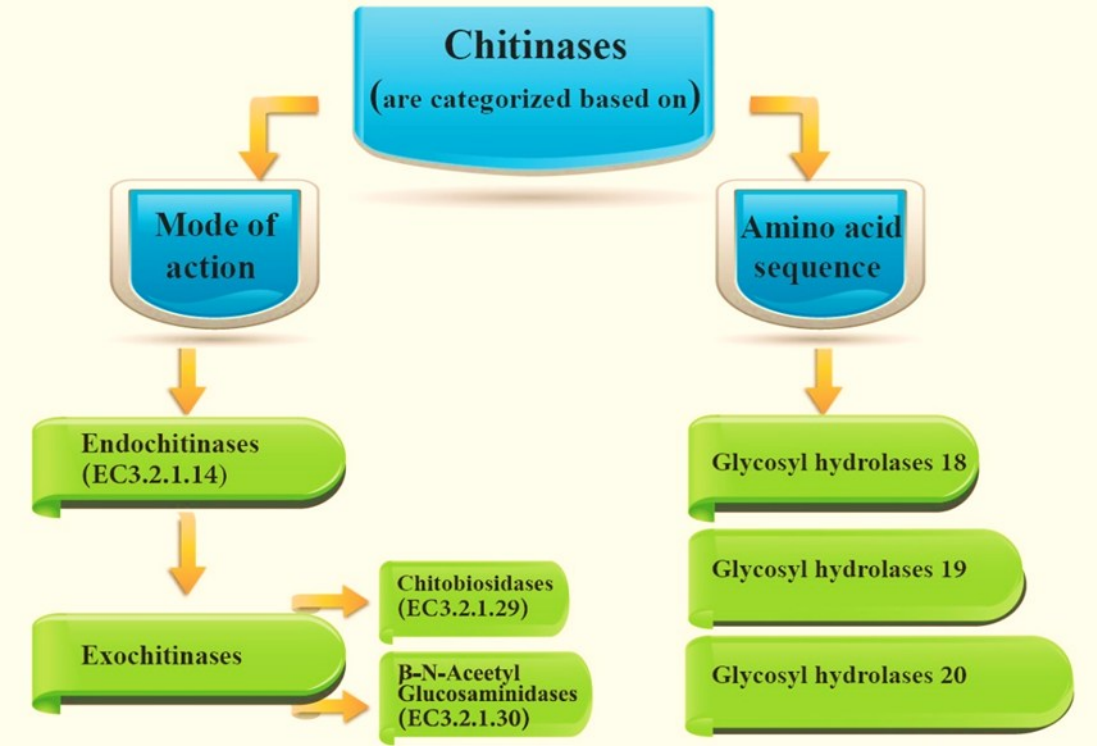

Fig. 2. Classification of chitinases according to amino acids sequences and mode of action. Adapted from (Funkhouser and Aronson 2007)

Chitinases cleave the β-1,4-glycosidic bonds in chitin by means of retaining or inverting mechanisms. Most GH18 chitinases follow a two-step retaining mechanism, using aspartic acid as a nucleophile and glutamic acid as an acid/base catalyst to maintain the anomeric configuration of the product. Endochitinases, such as SmChiA (GH18), are non-processive enzymes with a shallow, exposed substrate-binding cleft that enables

random internal cleavage; their catalytic site contains the conserved Asp-Glu-Asp motif (Horn *et al.* 2006). In contrast, exochitinases, including chitobiosidases, are processive enzymes with a tunnel-shaped active site that guides the chitin chain, allowing sequential release of disaccharide units without enzyme dissociation (Morimoto *et al*. 1997).

Chitinases are also classified into three glycosyl hydrolase (GH) families based on the sequences of their catalytic domains (Funkhouser and Aronson 2007): GH18, found in viruses, fungi, bacteria, insects, and mammals; GH19, present in actinobacteria, purple phototrophic bacteria, and plants; and GH20, associated with human chitinases that act on chitin degradation products rather than polymeric chitin (Fig. 2).

GH18 chitinases employ substrate-assisted catalysis, where the C2-acetamido group of GlcNAc acts as a nucleophile, forming a transient oxazolinium ion and retaining the stereochemistry of the glycosidic bond (Chen *et al*. 2020). GH19 chitinases use acid-base catalysis, where an acid protonates the glycosidic oxygen and a base attack the anomeric carbon, resulting in inversion of the anomeric configuration (Kawase *et al*. 2004). GH20 enzymes, including β-N-acetylhexosaminidases and chitobiases, hydrolyze chitobiose or N-acetylgalactos-amine from glyco-conjugates (Vaaje-Kolstad *et al*. 2013).

Chitinolytic Microorganisms

Microbial chitinases are generally preferred over their plant or animal counterparts in industrial and biotechnological applications for several key reasons. First, microorganisms can produce large amounts of chitinases at low production costs, especially when inexpensive and readily available substrates, such as seafood waste, are utilized (Kuddus 2014). Second, microbial chitinases exhibit stability across a wide range of temperatures and pH, making them suitable for diverse industrial applications (Al Abboud *et al*. 2022; Al-Rajhi, *et al*. 2023). Third, microorganisms can be genetically manipulated with relative ease to enhance chitinase productivity or enzymatic properties, opening avenues for the development of tailor-made enzymes for specific applications (Stoykov *et al*. 2014). Moreover, the extraction and purification of chitinases from microbial sources is generally more efficient and cost-effective compared to plant or animal sources (Oyeleye and Normi 2018). Chitinolytic microorganisms inhabit diverse terrestrial and aquatic environments, and shellfish waste. A straightforward approach to screen for these microorganisms involves culturing them on agar media containing colloidal chitin as the sole carbon source and identifying colonies by the formation of clearing zones around them (Abu-Tahon and Isaac 2020).

Bacterial chitinases

Bacterial chitin degradation is essential for biogeochemical cycling and sustaining ecosystem carbon–nitrogen balance (Kumar *et al.* 2022). Bacteria can sense chitin and respond through mechanisms such as movement toward the chitin source (chemotaxis) or growth in its direction (chemotropism). They also adhere to chitin surfaces, secrete extracellular chitinolytic enzymes to break down the polymer, and uptake the resulting chitin-derived oligosaccharides for metabolic use (Selenius *et al.* 2018).

The review emphasizes the considerable potential of bacterial chitinases for various commercial applications, owing to their stability under extreme pH and temperature conditions, rapid growth, and suitability for genetic engineering (Kumar *et al.* 2022). Chitinases are broadly occurring in *Arthrobacter*, *Aeromonas*, *Bacillus*, *Clostridium*, *Chromobacterium*, *Klebsiella*, *Pseudomonas*, *Serratia*, *Streptomyces*, and *Xanthomonas* (Jahromi and Barzkar 2018). From a functional and structural perspective, bacterial

chitinases are classified into three GH families: GH-18, GH-19, and GH-23 (Fig. 3), and the majority of bacterial chitinases belong to the GH-18 family (Udaya Prakash *et al.* 2010).

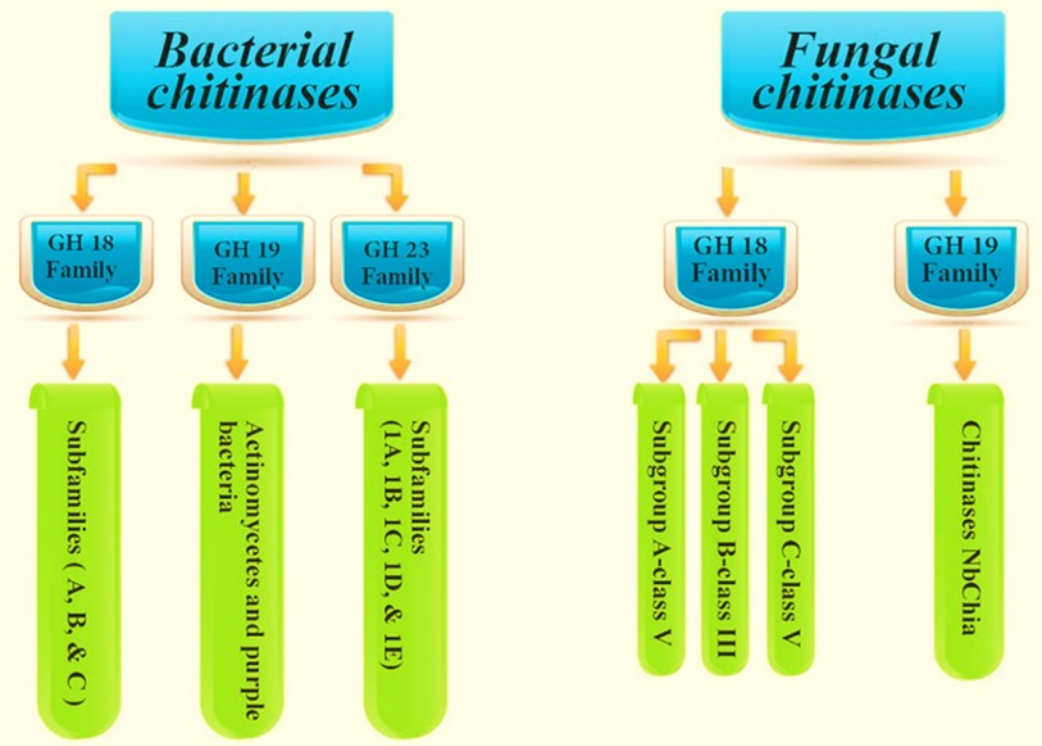

Fig. 3. Classification of bacterial and fungal chitinases

GH-18 bacterial chitinases are divided into three subfamilies, A, B, and C, based on sequence and structural features. Subfamily A, which is the most widespread, typically contains a chitin insertion domain (CID) that enhances binding to insoluble chitin, and generally functions as an endochitinase by randomly cleaving internal β-1,4-glycosidic bonds. Subfamily B, which lacks a CID, still contributes significantly to chitin hydrolysis and exhibits both endo- and exo-chitinase activities. Subfamily C is less well characterized and restricted to a limited number of bacterial species, indicating possible specialized adaptations.

Chitin-binding proteins (CBPs) enhance chitinase efficiency by promoting chitin degradation or binding to chitin-containing surfaces (Frederiksen *et al.* 2013). GH-19 chitinases are mainly distributed in actinobacteria and purple bacteria, whereas GH-23 chitinases are mainly present in peptidoglycan lyases from bacteria and bacteriophage. This group also includes goose-type (G-type) lysozymes, which are specifically active in the hydrolysis of chitin and chitooligosaccharides (Arimori *et al.* 2013). Several species noted for high chitinase production are listed in Table 1.

In nature, *Serratia marcescens* is among the most organized and efficient bacterial chitin degraders and has been extensively studied for its chitinase production (Vaaje-Kolstad *et al.* 2013). Multiple genes encoding chitinase have been identified in *Serratia marcescens* strains, whereas *the S. marcescens Nima* strain exhibits nearly 43-fold higher activity compared to the others (Bhattacharya *et al.* 2007).

Table 1. Bacterial Chitinases and their Classification

Microorganism	Gene/protein Name	Glycoside Hydrolase Family (GH)	Chitin Binding Protein (CBP)	Culture Components	Reference
Bacillus haynesii	-	-	-	Minimal media with glycerol (12%), yeast extract (0.2%), colloidal chitin (1%), and artificial sea water, 37 ºC, 48 h	Govindaraj *et al.* (2024)
Bacillus paralicheniformis	Chi23	GH-18	-	-	Xie *et al.* (2025)
Serratia marcescens	ChiB, and ChiC	GH-18		Luria Bertani broth	(Shrivastava *et al.* (2024)
Streptomyces spp.	-	-	-	Colloidal chitin broth medium, 35 °C, 148 h, submerged fermentation (SmF) at 120 rpm.	(El-Akshar *et al.* (2024)
Stenotrophomonas maltophilia	-	-	-	Colloidal chitin 1 %, pH 7, 37 ◦C 96 h, SmF	(Gonfa *et al.* (2023)
Enterococcus faecium	-	-	-	Whey/yeast extract medium pH 6.5. 37 °C, 24 h, Surface Fermentation (SF)	(Atwa *et al.(* 2022)
Pseudoalteromonas flavipulchra DSM 14401	ChiA, and ChiB	GH-18	-	Medium containing 0.05% peptone, 0.01% yeast powder, and chitin flake (3%)	(Ren *et al.* (2022)
Bacillus sp.	Chisb	GH-18	-	-	(Pan *et al.* (2019)
Bacillus thuringiensis	ChiA	GH-18	-	-	(Juárez-Hernández *et al.* (2019)
Bacillus thuringiensis B-387	-	-	-	Standard LB broth and in the medium with colloidal chitin, respectively	(Aktuganov *et al.* (2025)
Citrobacter freundii				Nutrient broth medium supplemented with 1%, 5%, and 10% wastes	(Taha *et al.* (2025)
Listeria monocytogenes	ChiA, and ChiB	GH-18	-	Brain heart infusion (BHI) broth, 37 ºC	(Halbedel *et al.* (2019)
Serratia marcescens	ChiA, ChiB, ChiC, and ChiD	GH-18	-	-	(Madhuprakash *et al.* (2019)
Streptomyces sp. F-3	ChiA, ChiB, ChiC, and Chi19A	GH-18, and GH-19	-	-	(Sun *et al.* (2019)

Microorganism	Gene/protein Name	Glycoside Hydrolase Family (GH)	Chitin Binding Protein (CBP)	Culture Components	Reference
Xenorhabdus nematophila HB310	Chi60, and Chi70		GH-18	-	(Liu *et al.* (2019)
Chitinolyticbacter meiyuanensis SYBCH1	Chi1	GH-18	-	Culture medium: glucose (0.4%), peptone (0.4%), yeast extract (0.4%), KH_2PO_4 (0.07%), K_2HPO_4 (0.03%), & $MgSO_4$ (0.05%), 37 °C, 12 h, SmF at 200 rpm.	Zhang *et al.* (2018)
Paenibacillus sp.	-	-	-	Minimal medium supplemented with shrimp waste (1%) & ammonium sulphate (0.5%), pH: 7.0, 37 ºC, 96 h, SmF.	Kumar*, et al.* (2018)
Salinivibrio sp.	ChiC	GH-18	-	Marine medium and colloidal chitin, 37 °C, 24 h, SmF.	Le and Yang (2018)
Streptomyces albolongus	ChiA	GH-18	-	Soluble starch (1%) & yeast extract (0.2 %), pH 7.3.	Gao *et al.* (2018)
Streptomyces thermodiastaticus	Chi 1	GH-18	-	Chitin powder (1.0%), yeast extract (0.1%), & $MgSO_4$ $7H_2O$ (0.03%), pH 7.0, 50 ºC, 120 h, , SmF at 100 rpm.	Take *et al.* (2018)
Paenibacillus sp. str. IK-5	ChiA, ChiB, ChiC and Chi19D	GH-18 and GH-19	-	-	Kusaoke *et al.* (2017)
Paenibacillus sp. FPU-7	ChiA, ChiB, ChiC, ChiD, ChiE, ChiF, and ChiW	GH-18	-	Peptone (1.0%) and NaCl (0.5%), pH 7.5, 30 ºC, SmF.	Itoh *et al.* (2013)
Serratia marcescens	ChiA, ChiB, and ChiC	GH-18	CBP21	-	Vaaje-Kolstad *et al.* 2013)
Serratia proteamaculans 568	ChiA, ChiB, ChiC, and ChiD	GH-18	CBP21, CBP28 and CBP50	-	Purushotham *et al.* (2012)
Bacillus thuringiensis	-	-	CBP 50	Luria-Bertani (LB)-media, 30 ºC	Mehmood *et al.* (2011)
Aeromonas schubertii	CHI53 CHI61	-	-	Luria-Bertani (LB) medium, chitin powder (2%), 28 ºC, 72 h	Liu *et al.* (2009)
Serratia marcescens	ChiA, ChiB, and ChiC,	GH 18	-	-	Horn *et al.* (2006)

Microorganism	Gene/protein Name	Glycoside Hydrolase Family (GH)	Chitin Binding Protein (CBP)	Culture Components	Reference
Streptomyces coelicolor	ChiA, ChiB, ChiC, Chi19F, and Chi19 G	GH 18 and GH 19	-	-	Kawase *et al.* (2006)
Alteromonas sp.	ChiA, ChiB, ChiC, and ChiD	GH18	CBP 1	Bacto Marine Broth, 27 °C, SmF	Tsujibo *et al.* (2002)
Burkholderia gladioli	ChiA 18, and ChiB 19	GH 18 and GH 19	-	Inorganic salts medium with colloidal chitin (0.15%), 30°C ,72 h	Kong *et al.* (2001)
Vibrio alginolyticus H-8	ChiC	GH 18	-	Medium containing squid chitin (0.5%), glucose (0.6%), peptone (0.75%), yeast extract (0.2%), & seawater (75%), 30 °C.	Ohishi *et al.* (1996)

Fungal chitinases

The chitinase enzyme is essential in the fungal life cycle, where it contributes to cell wall remodeling and plasticization, thereby regulating hyphal growth, tube extension, branching, fusion, germination, and division (Karthik *et al*. 2014; Bakri *et al*. 2022). The distribution and abundance of chitin differ among fungi; in filamentous species, it is mainly located in the inner cell wall layers adjacent to the plasma membrane, with a relatively high content of about 20%, whereas in yeasts, it is restricted to constriction rings, septa, and budding scars, where its content ranges from 0.5% to 5% (Hartl *et al*. 2012).

The regulation of fungal cell wall degradation, whether targeting self or non-self-structures, is thought to be governed more by substrate accessibility in healthy hyphae than by the specificity of chitinases. The susceptibility of the fungal cell wall to enzymatic hydrolysis is controlled by the balance between protection and deprotection during mycoparasitism, aging, and autolysis (Gruber and Seidl-Seiboth 2012). Fungi produce hydrophobic cell wall proteins such as QID74 and carbohydrate-binding proteins to shield their cell walls from the action of hydrolytic enzymes. *Trichoderma harzianum* produces a 74 kDa cell wall protein that is essential for both adherence to hydrophobic surfaces and mycelium protection (Rosado *et al*. 2007).

Fungal chitinases are traditionally categorized into classes III and V, based on their predominant occurrence in specific organisms (Fig. 3). Class III (plant-type) and Class V (bacterial-type) chitinases differ in their substrate-binding grooves: Class V enzymes possess deep, tunnel-shaped grooves and act as processive exo-chitinases, whereas Class III enzymes have shallow, open grooves and function as non-processive endo-chitinases (van Aalten *et al*. 2001; Hoell *et al.* 2005).

Based on the amino acid sequences of the GH18 family and the structure of their substrate-binding clefts, fungal chitinases are further divided into three subclasses: A, B, and C. Subgroups A and C belong to class V, while subgroup B belongs to class III (Gruber *et al*. 2011). Functionally, subgroup A chitinases are involved in fungal growth and autolysis, subgroup B chitinases are primarily nutritional enzymes in mycoparasites and insect-pathogen fungi, and subgroup C chitinases, found mainly in *Trichoderma atroviride* and *T. virens*, participate in both endogenous and exogenous chitin degradation (Berini *et al*. 2018; Hartl *et al*. 2012).

Several fungal genera, including *Aspergillus*, *Beauveria*, *Conidiobolus*, *Metarhizium*, *Mucor*, *Neurospora*, *Penicillium*, *Trichoderma*, and *Verticillium*, have been reported as potent producers of chitinases (Thakur *et al*. 2023). Some of the species reported to exhibit excellent chitinase activity are summarized in Table 2. Deng *et al*. (2019) reported that Chit-46 chitinase from *Trichoderma harzianum* suppresses the growth of the phytopathogenic fungus *Botrytis cinerea*, hydrolyzing chitin into $GlcNAc_2$ with up to 94.8% efficiency.

Optimization Parameters for Maximum Production of Microbial Chitinase

Environmental and nutritional conditions have a strong impact on microbial chitinases. These variables include the initial pH of the medium, the duration and temperature of the incubation, the various chitin sources, the impact of shaking velocity, and the effects of various carbon and nitrogen sources that were tested as salt basal media supplements (Tables 1 and 2). The primary factor influencing chitinase productivity is the form of chitin, such as crystalline chitin, shrimp or crab shell powder, or colloidal chitin. Colloidal chitin is a highly accessible form of chitin, ideal for chitinase studies. It is prepared from insoluble chitin powder, regardless of source. The process involves treating

chitin powder with a concentrated strong acid (*e.g.*, HCl) to break its crystalline structure. This acid-chitin mixture is then diluted with cold water, causing amorphous particles to precipitate, forming a colloidal suspension. Subsequent neutralization and washing remove residual acid and impurities. The final product is a milky, viscous suspension of amorphous chitin, offering increased surface area for enzymatic degradation (Abu-Tahon and Isaac 2020). Fungal chitinase production is typically performed by submerged fermentation, this method allows for better oxygen transfer, nutrient availability, and enzyme secretion into the liquid phase. It is widely used because it enables easy recovery and purification of chitinase from the culture broth (Abu-Tahon and Isaac 2020). In *Trichoderma viride*, maximum yields were obtained using colloidal chitin as the carbon source under optimized conditions of pH 6.5, 35 °C, and 125 rpm (Abu-Tahon and Isaac 2020).

Moreover, chitinase production is strongly influenced by incubation time, generally increasing to a peak before declining during prolonged cultivation. This pattern occurs because enzyme synthesis is closely linked to the microorganism's growth phase and metabolic activity, with maximum production typically observed during the logarithmic or early stationary phase. In addition, the availability of chitin as a substrate and the accumulation of metabolic byproducts significantly affect enzyme output, as nutrient depletion or the buildup of inhibitory compounds can reduce secretion or promote enzyme degradation (Karthik *et al*. 2014).

Shaking speed is a key factor influencing enzyme productivity, as mechanical forces can induce vacuolation in older hyphal compartments, potentially weakening the hyphae or promoting fragmentation (Paul *et al.* 1994). Chitinase production has been shown to vary with shaking velocities ranging from 100 to 200 rpm (Table 1 & 2), as agitation directly affects oxygen transfer, nutrient distribution, and shear stress in the culture; optimal shaking promotes sufficient aeration and mixing to enhance enzyme production, whereas too little or excessive agitation can reduce yield due to stress or limited substrate availability (Alves *et al.* 2018).

From a physiological standpoint, solid-state fermentation (SSF) provides several advantages for chitinase production, including high volumetric yields, elevated product concentrations, reduced effluent generation, and minimal requirements for sophisticated equipment. Moreover, solid substrates bound to amino acids are chemically more stable than free substrates, making them particularly suitable for large-scale production of economically valuable compounds (Stoykov *et al.* 2015). In this regard, El-Beltagi *et al*. (2022) reported that the ideal medium for chitinase production by *Talaromyces funiculosus* was crab shell chitin amended with yeast extract 0.2% and beet molasses 100% at pH 6.5 for seven days.

The One-Factor-At-a-Time (OFAT) approach involves varying a single factor while keeping all others constant. Although widely used, this method has notable limitations: it requires many experiments to assess multiple factors, it cannot reveal interactions between variables, it is time-consuming and costly, and it may miss the optimal combination of conditions (Vaidya *et al*. 2003). To address the limitations of the OFAT method, several statistical approaches have been developed, including Plackett-Burman design (PBD), central composite design (CCD), Taguchi's robust design (TRD), and response surface methodology (RSM) (Han *et al.* 2008). These methods offer simplicity, efficiency, and nutrient savings, while allowing the analysis of factor interactions, making them effective for optimizing enzyme production and media components (Mishra *et al.* 2012). For example, Lee and Kim (2015) optimized chitinase production in *Pseudomonas fluorescens* was achieved using PBD and CCD, identifying yeast extract, $CaCl_2.2H_2O$, and

crab shell powder, as key factors. The CCD-optimized medium increased enzyme activity to 1.03 U/mL, nearly 2.9 times greater than standard conditions.

Table 2. Chitinases Produced by Fungi and their Classification

Microorganism	Gene/ Protein Name	Glycoside Hydrolase Family (GH)	Culture Components	Reference
Irpex lacteus (white rot fungus)	Chi18C	GH18	Modified Mandel's medium containing wheat bran, 25 ºC SmF at 150 rpm, 144 h	Kamijo *et al*. (2025)
Aspergillus niveus	-	-	Minimal medium with 1% shrimp residues, pH 6.0, 30 ºC, 96 h SmF at 100 rpm.	Ornela Guimarães (2024)
Aspergillus niger			2 g of Molokhia stems were moistened with 10 mL of distilled water, 30 °C, 168 h under static conditions	Abdel Wahab *et al.*, (2023)
Cladosporium cladosporioides	-	-	Czapek-Dox broth medium containing chitin (2%), NaCl (10%), pH 6.6, 28 ºC, 192 h.	Al Abboud *et al.*, (2022)
Talaromyces funiculosus	-	-	Cab shell chitin amended with yeast extract (0.2%) and beet molasses (100%), pH 6.5, 168 h.	El-Beltagi *et al.* (2022)
Thermomyces lanuginosus	-	-	YPS growth medium containing yeast extract (0.4%), peptone (0.2%), and NaCl (2.5%), pH 6.5, 50 ºC, 48 h SmF at 150 rpm.	Suryawanshi Eswari(2022)
Trichoderma bissettii	-	-	Liquid basal media containing colloid-al chitin (1%), pH 6, 25 ºC, 336 h	Chung *et al.* (2022)
Penicillium ochrochloron	Poch1 & Poch11	- GH18	-	Wu *et al.* 2025)
Penicillium oxalicum	-	-	Medium containing powder chitin (3 %) and tryptone (4%),30 ºC, 72 h SmF at 200 rpm.	Xie *et al.* (2021)
Trichoderma viride	-	-	Colloidal chitin (1.4%) amended with maltose (1%) & yeast extract (1%), pH 6.5, 35 ºC, 96 h at 125 rpm.	Abu-Tahon and Isaac (2020)
Trichoderma harzianum	Chit46	GH18	-	Deng *et al.* (2019)
Xenorhabdus nematophila	Chi60 and Chi70	GH18	-	Liu *et al.*, (2019)
Coprinopsis cinerea	ChiE1	GH18	Rice straw medium containing rice straw (88%), bran (5%), corn meal (3%), 28 ºC	Zhou *et al.* (2018)
Gloeophyllum trabeum (brown rot fungi)	-	GH18	Sterilized wood blocks	Presley and Schilling. (2017).
Phanerochaete chrysosporium white Rot Fungus	Chi18D	GH18	Solid Hagem medium: Colloidal chitin (1%), K_2HPO_4 (0.03%), KH_2PO_4 (0.03%), $MgSO_4 \cdot 7H_2O$ (0.005%), NaCl (0.005%), agar (1.5%), pH 6.8–7.0	Karlsson *et al*.(2016)
Phellinus pini white Rot Fungus	-	-	**FR medium:** Glucose (2%), L-Asparagine (0.25%), D,L-Phenylalanine (0.015%), Adenine (0.00275%), Thiamine-HCl (50 µg/L), KH_2PO_4 (0.1%), $Na_2HPO_4 \cdot 2H_2O$ (0.01%), $MgSO_4 \cdot 7H_2O$ (0.05%), $CaCl_2$ (0.01%), $FeSO_4 \cdot 7H_2O$ (0.01%), pH ~5.0	Jaszek *et al*. (2014)
Beauveria bassiana	Chit1	GH18	-	Pinnamaneni *et al.* 2(010)
Metarhizium anisopliae	ChiA, ChiB, ChiC and ChiD	GH18	Minimum medium: Nacetyl-glucosamine (0.25%), $NaNO_3$ (0.6), & trace elements solutions, 28 ºC, 72 h, SmF at 180 rpm.	(Junges *et al.* (2014)

Microorganism	Gene/ Protein Name	Glycoside Hydrolase Family (GH)	Culture Components	Reference
Cladosporium sp.			Potato Dextrose medium, pH 6, 26 ºC, 120 h, SmF	Venkatachalam *et al.* (2015)
Ustilago maydis	Cts1, Cts2, and Cts3	GH18	Complete medium amended with glucose (1%), 28 °C, SmF at 200 rpm	Langner *et al.* (2015)
Aspergillus flavus	-	-	Wheat bran with chitin powder (1%), pH 6.4, 32 ºCm 190 h	Thadathil *et al.* 2014)
Aspergillus terreus	-	-	Shrimp-shell chitin powder amended with glucose (1%) and ammonium sulphate (1 %), pH 5, 30 ºC, 120 h, SmF at 120 rpm	(Aida *et al.* (2014)
Fusarium oxysporum	-	-	Wheat bran with chitin powder (1%), pH 6.4, 32 ºC, 167 h, SmF.	Thadathil *et al.* (2014)
Rhizopus oryzae	-	-	SIV broth with starch (2%) and urea (0.2), 30 ºC, 120 h, SmF at 120 rpm.	Chen *et al.* (2013)
Aspergillus niger	-	-	Medium with yeast extract (0.15%) and colloidal chitin (2%), pH 6.5, 26 ºC, 240 h, SmF at 100 rpm.	Brzezinska and Jankiewicz (2012)
Gliocladium catenulatum	-	-	Potato dextrose medium, pH 4.5, 30 ºC, 24–30 h	Ma *et al.* (2012)
Penicillium monoverticillium	-	-	Wheat bran amended with 1% shrimp chitin powder (1%), pH 6.4, 30 ºC, 166 h	Suresh and Anil Kumar (2012)
Saccharomyces cerevisiae	Cts1	GH18	-	Hurtado-Guerrero and van Aalten (2007)

Purification and Characterization of Microbial Chitinases

Enzyme purification involves sequential steps aimed at isolating the enzyme from complex mixtures while preserving its activity. The purification and characterization of microbial chitinases are crucial for determining their specific biochemical properties, such as substrate specificity, optimal pH, and thermal stability. This knowledge is essential for harnessing their potential in various biotechnological applications, including biocontrol of plant pathogens, waste management, and the production of valuable chitooligosaccharides (Govindaraj *et al*. 2024). Various techniques have been employed for chitinase purification, typically including dialysis, precipitation using ammonium sulfate or organic solvents, gel filtration chromatography, and ion-exchange chromatography. The final assessment of enzyme purity and homogeneity is typically conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Table 3). The final and most critical assessment of enzyme purity and homogeneity is typically conducted using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). This technique is considered the gold standard because it separates proteins based solely on their molecular weight, independent of their native charge or conformation. The successful outcome of the purification process is visually confirmed by the appearance of a single, sharp band on the gel, which corresponds precisely to the expected molecular weight of the target enzyme, thereby providing unambiguous evidence that all contaminating proteins have been effectively removed (Chen *et al*. 2013).

Table 3. Purification and Characterization of Chitinases Produced by Different Microorganisms

Organism	Enzyme Purification Procedures	Substrate of Assay and Molecular Weight (kDa)	Specific Activity (U/mg) and Purification Yield (%)	Optimum pH and Temp.	K_m (mg/mL) V_{Max} (mm/min/mg)	Inhibitor	Inducer	Reference
Aspergillus niveus	Sephadex G-100	4-Nitrophenil-N-acetyl-β-D-glucosaminide (pNP-GlcNAc), 49.3	44.3% and 14.3%	6.0 and 65 ºC	Km of 2.67 & V_{max} of 12.58	EDTA, β-Mercapto-ethanol	-	Ornela and Guimarães (2024)
Bacillus haynesii	30 kDa and 10 kDa filters	Colloidal chitin, 35	3.49%, and 34.7%	6.0 and 37 ºC	K_m of 0.01 & V_{max} of 191	-	Mn^{2+}	Govindaraj *et al.* (2024)
Aspergillus niger	-	pNP-GlcNAc	-	5.0 and 60 ºC	K_m of 2.67 0.78	Co^{2+}, Cu^{2+}, Hg^{2+}, & Zn^{2+}	Na+	Abdel Wahab *et al.* (2023)
Talaromyces funiculosus	$(NH_4)_2SO_4$ (60%), Sephadex–G100 & DEAE cellulose	Colloidal chitin, 45	9.32%, and 60.8%	6.5 and 40 ºC	-	Hg^{2+}, Ag^{2+}, Li^{+}, Zn^{2+}, & Co^{2+}	Ca^{2+}, Cu^{2+}, Na^{+}, Mn^{2+}, and Mg^{2+}	El-Beltagi *et al.* (2022)
Streptomyces albus	$(NH_4)_2SO_4$ (60%), DEAE-cellulose column	Colloidal chitin	0.848%, and 4.4%	6.0 and 30 ºC	-	Zn^{2+}	Mn^{2+}, K^{+}, Na^{+}, Mg^{+}, Fe^{2}, Ca^{2+}	Ekundayo *et al.* (2022)
Trichoderma viride AUMC13021	$(NH_4)_2SO_4$ (65%), Sephadex G-100m & DEAE-Cellulose	Colloidal chitin, 62	210%, and 73.1%	6.5 and 40 ºC	K_m value of 6.66 & V_{max} of 90.8	Hg^{2+}, Zn^{2+}, dodecyl sulphate, & EDTA	Ca^{2+} and Mn^{2+}	Abu-Tahon and Isaac (2020)
Aeromonas sp.	$(NH_4)_2SO_4$ (70%) DEAE-cellulose Sephadex G-50)	Colloidal chitin, 53	27.81%	6.5 and 55 ºC	K_m value of 0.64 V_{max} of 2.3	Hg^{2+}, Mg^{2+}, Br^{3+} and Ag+	Cu^{2+}, Co^{2+}	Jahangiri *et al.* (2019)

Organism	Enzyme Purification Procedures	Substrate of Assay and Molecular Weight (kDa)	Specific Activity (U/mg) and Purification Yield (%)	Optimum pH and Temp.	K_m (mg/mL) V_{Max} (mm/min/mg)	Inhibitor	Inducer	Reference
Aspergillus niveus	$(NH_4)_2SO_4$ (80%), Sephadex G-100	Colloidal chitin, 44	13.03%, and 40%	5 & 65 ºC	K_m value of 3.51 V_{max} of 9.68	KI, $CuSO_4$, $ZnSO_4$	$MnCl_2$	Alves *et al.* (2018)
Actinomyces griseoaurantiacus	$(NH_4)_2SO_4$, DEAE-cellulosem & Sephacryl S-300	Colloidal chitin, 130	93.75%, and 17.6%	4.5 & 40 ºC	K_m value of 0.22 V_{max} of 19.6	Fe^{2+}, & Cu^{2+}	Mn^{2+} , & Zn^{2+}	Shehata *et al.* (2018)
Humicola grisea	CM-sepharose & DEAE-sepharose	Colloidal chitin, 50	9.09%, and 17.06%	3 & 70 ºC		Hg^{2+}, Cu^{2+}, & EDTA	Mn^{2+}, Co^{2+}, & NH^{+}_4,	Kumar, Brar, *et al.* (2018)
Stenotrophomonas maltophilia	$(NH_4)_2$ SO_4, & sephadex G -100	Colloidal chitin, 50	1.5%	6.5 & 37 ºC		EDTA	$CaCl_2$	Shaikh *et al.* (2018)
Aspergillus terreus	$(NH_4)_2SO_4$ (65%) Sephadex G-100, & DEAE-sephadex A-50	Colloidal chitin, 60	182.1%, and 12%	5.6 & 50 ºC	-	Cd^{2+},Zn^{2+}, pb^{2+}, Hg^{2+}	Ca^{2+}, Mn^{2+}, & Na^{2+}	Farag *et al.* (2016b)
Paenibacillus barengoltzii CAU904	$(NH_4)_2SO_4$	Colloidal chitin, 67	2.34%, and 8%	3.5 & 60 ºC	K_m value of 3.35	Hg^{2+}, Ag^{+}, $Ni2^{+}$, & Cr^{2+}	Na^{+},Ca^{2+}, & Mg^{2+}	Fu *et al.* (2016)
Bacillus pumilus JUBCH08	$(NH_4)_2SO_4$ (70% w/v), & sephadex G-200	Colloidal chitin, 64	2.51%, and 74.7 %	8 & 70 ºC	K_m value of 0.13 V_{max} 38.23	Fe^{3+}, Ag^{+}, Hg^{2+}	Mg^{2+}, Co^{2+}, Ca^{2+}, & Mn^{2+}	Bhattacharya *et al.* (2016)
Verticillium lecanii	$(NH_4)_2SO_4$ (90%), phenyl-sepharose, & DEAE-sepharose,	Colloidal chitin, 42	29.26%, and 16.9%	4.6 & 40 ºC	-	Cu^{2+}, K^{+}, Na^{+}	Mg^{2+}	Yu *et al.* (2015)

According to the literature, Sephadex is the most used gel filtration medium for chitinase purification, while diethylaminoethyl cellulose (DEAE) is frequently utilized for ion exchange chromatography, as shown in Table 3. In this respect, Abu Tahon and Issac (2020) reported that chitinase from *Trichoderma viride* was purified to homogeneity with a 73.1% yield and a 5.48-fold purification using ammonium sulfate precipitation (65%), Sephadex G-100, and DEAE-cellulose. The pure enzyme recorded a molecular mass of 62 kDa, exhibited maximal activity at pH 6.5 and 40 °C, and was activated by Ca^{2+} and Mn^{2+}, while Hg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, dodecyl sulfate, and EDTA inhibited its activity. Colloidal chitin was the preferred substrate, with an apparent Michaelis constant (K_m) of 6.66 mg/mL and maximal velocity (V_{max}) of 90.8 U/mL

Applications for Chitinases

Chitinases are versatile enzymes. They have numerous applications such as agricultural applications, medical applications, as well as biotechnological applications. Those applications will be discussed in detail.

Agriculture applications

In agriculture, chitinases are being used because they exhibit a combating role against pathogenic chitin-containing organisms like fungi, insects, and the eggshells of plant-parasitic nematodes (Malik *et al*. 2022). It was suggested that chitinases are used as fungal antagonists, confirming their important role as biocontrol agents against fungal plant diseases. Chitinases break down the cell wall of fungi, which is composed of chitin, glucan, and wall proteins (Abdelraouf *et al*. 2024). They also damage pathogen conidial germination, germ tube elongation, and can damage oospores. Additionally, other effects of chitinases were observed as deformities in the fungal cellularity, damage of the protoplasm, mycelial distortion and lysis, and changing the membrane permeability leading to leakage of intracellular contents (Awad *et al*. 2017). Table 4 presents a list of various microbial chitinases that have been reported to demonstrate fungicidal, insecticidal, and nematocidal activities. For instance, chitinase derived from *Streptomyces enissocaesilis* and *S. rochei* showed antifungal effect against the causal agents of *Fusarium* wilt (*Fusarium oxysporum*) and damping-off disease (*Rhizoctonia solani*) (El-Akshar *et al*. 2024).

Moreover, the growth of the human (opportunistic) pathogens *Candida* species, *Aspergillus fumigatus*, and *Cryptococcus neoformans* was inhibited by the chitinase produced by *Trichoderma viride* (Abdelraouf *et al*. 2024). It is known that invertebrate animal species contain chitin in their exoskeleton, tracheal system, epidermal cuticle, and the eggshell of the nematodes. Hence, chitinases can be used as insecticides and pesticides.

Chitinases are indispensable for biological control because their hydrolytic activity targets chitin, the second most abundant biopolymer, which is integral to the structural integrity of various pests. Specifically, by degrading chitin—a key component of fungal cell walls, insect cuticles, and the peritrophic matrix—chitinases effectively induce the lysis of pathogenic fungi and disrupt essential insect processes like metamorphosis and gut function. This mechanism establishes chitinases as potent, environmentally sound biopesticides and biocontrol agents (Navarro-González *et al.* 2019). It was found that *Penibacillus* sp. effectively controlled *Helicoverpa armigera* larvae by reducing the feeding rate and body weight, which subsequently increased the rate of larval mortality (Singh *et al*. 2016). Additionally, *Stenotrophomonas* and *Chromobacterium* were found to suppress the cyst nematode *Globodera rostochiensis* (Iqbal and Anwar 2019).

Table 4. Antifungal, Insecticidal, and Nematocidal Actions of Microbial Chitinases

Chitinases Producing Microbes	Activity	Target Fungal Species	References
Streptomyces enissocaesilis, & S. rochei	Fungicidal	*Fusarium solani, & Rhizoctonia solani*	(El-Akshar *et al.* (2024)
Bacillus subtilis	Fungicidal	*Aspergillus niger, & Rhizoctonia solani*	(Shafiq *et al.* (2024)
Streptomyces griseus, & Trichoderma viride	Fungicidal	*Aspergillus fumigatus, Cryptococcus neoformans, & Candida species*	(Abdelraouf *et al.* (2024)
Alcaligenes faecalis	Fungicidal	*Colletotrichum gloeosporioides*	(KM *et al.* (2024)
Aeromonas sp.	Fungicidal	*Fusarium solani, Alternaria alternata, & Botrytis cinerea*	(Cadirci and Yilmaz (2023)
Burkholderia gladioli	Fungicidal	*Ustilaginoidea virens, Alternaria solani, Fusarium oxysporum,*	(Yang *et al.* (2023)
Streptomyces albus, & S. flavogriseus,	Fungicidal	*Fusarium graminearum, Rhizoctonia solani, & Botrytis cinerea*	(Abo-Zaid *et al.* 2021)
Bacillus cereus, & B. subtillis,	Fungicidal	*Colletotrichum sp., Rhizoctonia sp., & Fusarium sp.*	(Malik *et al.* (2022)
Bacillus velenzensis	Fungicidal	*Fusarium fujikuroi, F. graminearum, & Alternaria alternata*	(Kim *et al.* (2022)
Streptomyces albus, & S. flavogriseus	Fungicidal	*Aspergillus niger, A. oryzae*	(Umar *et al.* (2021)
Paenibacillus sp.	Fungicidal	*Fusarium oxysporum, Alternaria burnsii, & Rhizoctonia solani*	(El-Sayed *et al.* (2019)

Chitinases Producing Microbes	Activity	Target Insect Species	References
Aspergillus niger	Insecticidal	*Galleria mellonella*	(Abdel Wahab *et al.* (2023)
Cladosporium cladosporioides	Insecticidal	*Culex pipiens*	(Al Abboud *et al.* (2022)
Xenorhabdus nematophila	Insecticidal	*Helicoverpa armigera*	Mahmood *et al.* (2020)
Penicillium chrysogenum	Insecticidal	*Culex pipiens*	H Mansour *et al.* (2019)
Penicillium ochrochloron	Insecticidal	*Galleria mellonella*	Wu *et al.* (2025)
Bacillus pumilus	Insecticidal	*Scirpophaga incertulas*	Rishad *et al.* (2017)
Bacillus thuringiensis	Insecticidal	*Plutella xylostella*	Avupati *et al.* (2017)
Planomicrobium sp.	Insecticidal	*Tribolium castaneum*	Tawfiq *et al.* (2025)
Aspergillus awamori	Insecticidal	*Galleria mellonella, Spodoptera littoralis, & Agrotis ipsilon*	Awad *et al.* (2017)
Chitinases Producing Microbes	**Activity**	**Target Nematode Species (stage)**	**References**
Stenotrophomonas, & *Chromobacterium*	Nematocidal	*Globodera rostochiensis*	Iqbal and Anwar (2019)
Bacillus thuringiensis	Nematocidal	*Caenorhabdi*	Qin *et al.* (2016)
Paenibacillus sp.	Nematocidal	*Helicoverpa armigera*	Singh *et al.* 2016)
Duddingtonia flagrans	Nematocidal	*Cyathostomin infective larvae*	Braga *et al.* (2015)
Pseudomonas fluorescens	Nematocidal	*Meloidogyne incognita* (egg)	Lee and Kim (2015)

Pharmaceutical applications

Chitinases are characterized by their potent antifungal, antibacterial, and antioxidant properties. Hence, their dominant usages in various medical applications are apparent. Because chitin is a main component of fungal cell walls, chitinase effectively acts as a biocontrol agent by breaking down the cell wall of pathogenic fungi, whether they are active or not (Halder *et al*. 2013). Chitinase causes damage and deformity of the hyphae and spores by disrupting the fungal cell walls, which causes mycelial lysis (Halder *et al.* 2013, Al-Rajhi *et al*. 2022). Chitinases of microbial origin can degrade chitin into chitosan-oligosaccharides with positive charges, which enable them to attack the negatively charged bacterial cell wall, causing its damage, increasing its permeability, leaking of the bacterial cell components, and finally, death of the bacterial cell occurs (Shehata *et al.* 2018).

The enhanced antimicrobial efficacy of polycationic chitosan against Gram negative bacteria is fundamentally driven by structural differences in the bacterial cell wall. Chitosan is positively charged due to its free amino groups, enabling strong electrostatic interactions. The Gram negative bacteria surface, characterized by lipopolysaccharides (LPS), carries a high overall negative charge. This strong attraction between positive chitosan and negative LPS leads to the crucial disruption and permeabilization of their outer membrane. This breach allows the chitosan to penetrate the cell, resulting in the leakage of intracellular contents and subsequent cell death. In contrast, Gram +ve bacteria, while also negatively charged, possess a significantly thicker, rigid peptidoglycan layer. This robust physical barrier effectively hinders the access and disruptive action of chitosan, rendering Gram positive bacteria less susceptible to the compound (Olicón-Hernández *et al*., 2015). Table 5 presents the various medical applications of microbial chitinases, including their potential roles as antifungal, antibacterial, anticancer, and antioxidant agents. Chitinases produced by *Bacillus haynesii* exhibited antifungal activity against *Fusarium oxysporum* and *Penicillium chrysogenum*, with mean inhibition zones of 33 mm and 12 mm in diameter, respectively (Govindaraj *et al.* 2024).

Similarly, microbial chitinases attain their anticancer potential from their ability to interact with cancer-specific polysaccharides containing compounds such as glycoproteins or glycolipids, which are located on the surface of the tumor cells and break down their carbohydrate moieties, causing functional damage and tumor cell death (Pan *et al.* 2005). However, the precise mechanism of inhibiting the proliferation of cancer cells remains unknown. Some explanations attributed such effects on the proliferation of the cancer cells to the differences in the electrostatic chargers of chitosan-oligosaccharides that may lead to increased cell permeability as above-described and/or alteration of the factor expressions of tumor cells (Liaqat and Eltem 2018). Many lines of cancer cells are being influenced by microbial chitinases, such as breast, lung, colon, bladder, and melanoma (Pan 2012). IC50 values of ChiB and ChiC from *Serratia marcescens* were found to be 4.63 µM and 2.36 µM, respectively, for the MCF-7 cells (Shrivastava *et al.* 2024).

Synthesis of antioxidants with potential scavenging effects to eliminate free radicals is accompanied by adverse effects, hence the importance of applying novel biological strategies for reactive oxygen species (ROS) scavenging, chelation of transition metals, and detoxification of antioxidants for free radical elimination (Halder *et al.* 2013). Chitooligosaccharides, produced by enzymatic hydrolysis of chitin and chitosan, display potent antioxidant activity (Khalil *et al*. 2017). Their antioxidant effects are attributed to hydroxyl and amino groups, which interact with unstable free radicals, converting them into molecular radicals (Halder *et al*. 2013). Purified chitinase from *Talaromyces*

funiculosus achieved maximum inhibition of DPPH and ABTS radicals at approximately 57.8% and 63.7%, respectively (El-Beltagi *et al*. 2022).

Table 5. Medical Applications of Microbial Chitinases

Producer	Activity	Target	Finding	Reference
Marine Bacillus haynesii	Antifungal	*Fusarium oxysporum* and *Penicillium chrysogenum*	Chitinase resulted in inhibition zones with diameters of 33 mm against *Fusarium oxysporum* and 12 mm against *Penicillium chrysogenum.*	Govindaraj *et al.* (2024)
Aspergillus niveus	Antifungal	*Trichoderma harzianum* and *Penicillium purpurogenum*	The chitinase inhibited the growth of *Trichoderma harzianum* with a minimum inhibitory concentration (MIC) of 22.4 µg/mL and *Penicillium purpurogenum* with a MIC of 11.2 µg/mL.	Ornela and Guimarães (2024)
Streptomyces enissocaesilis & *Streptomyces rochei*	Antifungal	*Fusarium solani* and *Rhizo ctonia solani*	Chitinases produced by *Streptomyces enissocaesilis* and *Streptomyces rochei* inhibited the mycelial growth of *Fusarium solani* and *Rhizoctonia solani* by 88% and 86%, respectively. .	El-Akshar *et al.* (2024)
Serratia marcescens	Anticancer	Breast cancer cell line (MCF-7).	The IC50 value of chitinase ChiB against MCF-7 cells was approximately twice as high (4.63 µM) as that of ChiC (2.36 µM)).	Shrivastava *et al.* (2024)
Aspergillus niger	Antifungal	*Candida albicans*	Chitinase produced a 30 mm zone of inhibition against *Candida albicans*.	Abdel Wahab *et al.* (2023)
Amanita sp.	Antifungal	*Alternaria alternata*	Chitinase produced by *Amanita* sp. inhibited the mycelial growth of *Alternaria alternata* by approximately 45% and 48% at 50 °C using dead fungal mycelia and chitin as substrates, respectively, while inhibition decreased to 20% and 22% at 60 °C.	Al-Rajhi *et al.* (2023)
Trichoderma bissettii	Antifungal	*Aspergillus flavus* and *Aspergillus niger*	Crude chitinase showed inhibitory activity against hyphal growth of *Aspergillus flavus* and *Aspergillus niger*	Chung *et al.* (2022)
Cladosporium cladosporioides	Antifungal and insecticidal activity	*Curvularia lunata* and *Fusarium oxysporum*	Chitinase (100 U/ml) reduced the growth of *Curvularia lunata* by 51.3% and *Fusarium oxysporum* by 51.67%.	Al Abboud *et al.* (2022)
Talaromyces funiculosus	Antibacterial and antifungal	*Pseudomonas aeruginosa, Escherichia coli*), *(Bacillus subtilis, Staphylococcus aureus*), and *Aspergillus niger,*	Purified chitinase demonstrated antimicrobial activity against *P. aeruginosa, B. subtilis, A. niger, C. albicans*, and *E. coli*, with inhibition zone diameters of 38, 29, 26, 25, and 24 mm, respectively	El-Beltagi *et al.* (2022)

Producer	Activity	Target	Finding	Reference
		Candida albicans.		
Talaromyces funiculosus	Anticancer	MCF-7	A concentration of purified chitinase (1000 µg/mL) induced higher toxicity in the cancer cell lines MCF7 (97%), HCT116 (88.2%), and HepG2 (97.1%).	El-Beltagi *et al.* (2022)
Talaromyces funiculosus	Antioxidant	DPPH &ABTS	Purified chitinase (400 µg/mL) resulted in maximum inhibition of approximately 57.8% for DPPH and 63.7% for ABTS.	El-Beltagi *et al.* (2022)
Penicillium oxalicum k10	Antifungal	*Sclerotinia sclerotiorum* and *Mucor circinelloides*	Chitinase prevented the mycelial growth of the phytopathogenic fungi *S. sclerotiorum* and *Mucor circinelloides*	Xie *et al.* (2021)
Trichoderma viride	Antifungal	*Fusarium oxysporum* f. sp. *lycopersici race* 3 the causal agent of tomato wilt.	Purified chitinase demonstrated a 45% inhibition of *Fusarium oxysporum f.* sp. *lycopersici race 3* hyphal growth.	Abu-Tahon and Isaac (2020)
Trichoderma viride	Anticancer	MCF7 and colorectal carcinoma cell line (HCT-116)	Purified chitinase has a toxic effect on MCF7 with an IC50 value 20 mg/mL, and HCT-116 cell lines with an IC50 value 44 mg/mL	Abu-Tahon and Isaac (2020)
Penicillium chrysogenum	Antifungal	*Penicillium digitatum* and *Penicillium italicum*	Partially purified chitinase significantly reduced the linear mycelial growth of *P. digitatum* by 70% and *P. italicum* by 72.2%.	Atalla *et al.* (2020)
Streptomyces halstedii H2	Antioxidant	DPPH	The crude chitinase exhibited a maximum DPPH inhibition of 84%.	Shalaby *et al.* (2019)
Aeromonas spp.	Anticancer	MCF-7 & prostate cancer cell line (PC-3)	Purified chitinase has a toxic effect to MCF7 with an IC50 value 300 µg/ml, and (PC-3) cell lines with an IC50 value 400 µg/mL	Hashim and Nema (2018)
Aspergillus grise-oaurantiacus	Antibacterial	Gram positive bacteria	Chitinase produced a 22 mm zone of inhibition against Gram positive bacteria	Shehata *et al.* (2018)
Aspergillus niveus	Antifungal	*Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. phoenicis*, and *Paecilomyces variotii*	Chitinase exhibited antifungal activity against *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus phoenicis*, and *Paecilomyces variotii*, with minimum inhibitory concentrations (MICs) of 84, 21, 24, 24, & 21 µg/mL, respectively.	Alves *et al.* (2018)
Aspergillus grise-aurantiacus	Antioxidant	DPPH	Chitinase exhibited a maximum DPPH inhibition of 60% at 24 h incubation time.	Shehata *et al.* (2018)

Producer	Activity	Target	Finding	Reference
Aspergillus terreus	Antifungal and Antibacterial	*Aspergillus niger, Aspergillus oryzae, Penicillum oxysporium, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa,*	The purified chitinase exhibited potent inhibitory activity against *A.niger, A. oryzae, P. oxysporium, S. aureus, S. typhi, & P. aeruginosa* with inhibition zone diameters of, 22, 18, 17, 15, & 14 mm, respectively.	Farag *et al.* (2016a)
Bacillus cereus	Antioxidant	DPPH, hydroxyl radical and ABTS.	Culture supernatant caused the maximum inhibition percentage of DPPH, hydroxyl radical and ABTS to be about 83%, 99.7%, & 51%, respectively.	Azam *et al.* (2014)

Industrial and Environmental Applications

Protoplast release

Fungal cell wall degradation and protoplast formation are primarily mediated by microbial enzymes. Although non-enzymatic and mechanical methods have also been reported, their practical applications remain limited (Sun *et al*. 1992). Microbial protoplasts serve as important tools in biochemical, genetic, and physiological studies (Hassan 2014) and are particularly useful for investigating enzyme localization in fungi (Sonawane *et al*. 2016). Advances in protoplast fusion have enabled genetic manipulation, allowing the combination of genes from different organisms, which facilitates strain improvement, enhances genetic recombination, and contributes to the development of industrially valuable strains (Patil *et al*. 2013).

Chitinase plays a key role in releasing protoplasts from microbial species whose cell walls contain substantial amounts of chitin. Crude chitinase extracted from *Rhizopus stolonifer* generated protoplasts of *Aspergillus niger, Aspergillus oryzae*, *Fusarium moniliforme*, and *Trichoderma viride* (Sonawane *et al.* 2016). In another study, *Penicillium ochrochloron* chitinase demonstrated high efficacy in generating protoplasts from *Aspergillus niger* (Patil *et al.* 2013). Due to the complex composition of chitin and glucans in fungal cell walls, protoplast formation necessitates a mixture of lytic enzymes, since single enzymes show limited activity (Sonawane *et al.* 2016). In this context, the combination of purified chitinase A from *Streptomyces cyaneus* with α-1,3-glucanase from *Bacillus circulans* KA-304 exhibited enhanced protoplast formation activity (Yano *et al.* 2008). Likewise, chitinase and β-glucanase enzyme complexes demonstrated great protoplast-forming efficiency between *Trichoderma harzianum* and *T. viride* (Hassan 2014).

Production of single cell proteins

A substantial amount of chitinous shellfish waste is generated. Thus, seafood waste, which is a byproduct of the shellfish processing industry, is considered a significant challenge (Nirmala 1991). Among the shell waste, Crustacean shell consists of 30 to 40% proteins, 30 to 50% calcium carbonate, and 20 to 30% chitin (Kurita 2006).

Single-cell protein (SCP) represents a valuable protein source and is considered an alternative to fish and soybean meals (Le and Yang, 2019). Chitinase plays a vital role in the production of single-cell proteins SCP by hydrolyzing chitin, which is abundant in

shellfish and other chitinous waste, into soluble chitooligosaccharides (COS) and monomeric sugars, primarily N-acetylglucosamine (GlcNAc). The resulting COS serve as readily assimilable carbon and nitrogen sources for various microorganisms, including yeasts and bacteria, which further hydrolyze them into monomeric sugars. These monosaccharides are then metabolized to produce microbial biomass rich in proteins, forming SCP. This enzymatic process provides a sustainable and efficient pathway to convert chitinous waste into high-value protein, supporting the development of a circular bioeconomy (Le and Yang 2019). Chitinases from *Penicillium ochrochloron* hydrolyzed chitin into GlcNAc, which was then utilized as a substrate for SCP production by *Yarrowia lipolytica* (Le and Yang 2019). Chitin was enzymatically hydrolyzed by *Serratia marcescens* QMB1466 chitinase to generate a hydrolysate, later used for yeast single-cell protein production (Revah and Carroad 1981). The SCP is produced from fungal sources, for example, *Candida tropicalis*, *Myrothecium verrucaria*, *Hansenula polymorpha*, and *Saccharomyces cerevisiae*, which is a major source of chitinase production with over 60% SCP and low nucleic acid contents (Dahiya *et al.* 2006). Moreover, the generation of SCP using *Penicillium ochrochloron* was also previously reported (Patil 2014).

Production of chitooligosaccharides

Hydrolysis of chitin by chitinases produces small chitooligomers and chitooligosaccharides (COS), which have diverse applications in agriculture, medicine, pharmaceuticals, and the food industry. COS are insoluble in propanol, ethanol, acetone, ethyl acetate, and butanol, partially soluble in dimethyl sulfoxide (DMSO) and methanol, and fully soluble in water (Liang *et al.* 2018). In the food industry, COS are used to enhance product quality and as dietary supplements to boost immunity (Martinez *et al.* 2012). Incorporation of COS into chitosan films for food packaging has been shown to improve antimicrobial properties (Fernandez-de Castro *et al.* 2016). In the human colon, specific bacteria degrade COS into short-chain fatty acids and other beneficial metabolites, providing probiotic effects (Selenius *et al.* 2018), while daily administration of 100 mg/kg COS increases Bifidobacterium populations and decreases *E. coli* levels (Wan *et al.* 2017).

Pharmaceutical applications of chitinases include antihypertensive, antioxidant, antitumor, wound-healing, antiallergic, and hypocholesterolemic effects, making them suitable for drug delivery, disease treatment, and the production of implants and surgical materials (Rameshthangam *et al.* 2018). COS also exhibit potent antimicrobial activity against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Staphylococcus xylosus*, *Bacillus cereus*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, and *Proteus vulgaris* (Castañeda-Ramírez *et al.* 2013).

COS shows potent antifungal effects against *Aspergillus, Candida, Saccharomyces*, and *Trichophyton* (Muanprasat and Chatsudthipong 2017). These molecules can reduce colonic mucosal inflammation through various mechanisms. They may increase malondialdehyde levels and enhance nitric oxide synthase activity, while decreasing catalase and glutathione levels, and modulating the TNF-α pathway (Bekale *et al.* 2015). Additionally, COS showed cytotoxic effects against A549 and HCT-116 cell lines, with in vitro IC_{50} values of 48.6 µg/mL and 1329.9 µg/mL, respectively. *In vivo* studies in mice revealed a tumor inhibition percentage of up to 58.5% (Zou *et al.* 2016). In the agricultural field, fungicidal and bactericidal properties against phytopathogens were observed. Additionally, they were used as plant growth regulators, immune boosters, and to improve the tolerance of plant seedlings to salt, heat, and cold stress (Zhang *et al.* 2019). An increase in the level of the IAA hormone in *Brassica napus* was seen after treatment with hetero

COS, resulting in enhancement in the height of the plant, the number of branches as well as plant biomass. The plant agronomic properties and upregulation of the main genes controlling the signaling pathway were improved by COS treatment (Tang *et al.* 2022). The germination of wheat seeds was reported to be prompted by COS (Fu *et al.* 2019). Similarly, the level of glutamate and proline, which contribute to powerful plant growth and cold tolerance enhancement of rice seedlings, was increased by COS treatment (Zhang *et al.* 2019).

Dye removal

Several chitin-synthesizing microorganisms have been investigated for their dye-removal potential. An innovative and powerful biocomposite absorbent was created by *Bacillus subtilis* through the bacterial biomass-mediated modification of chitosan. Such absorbents showed a high efficiency in removing the toxic dye of the textile Reactive Orange 16 in aqueous solution (Agha *et al.* 2025). Similarly, the efficiency of the Brilliant Blue dye removal was improved by *Aspergillus niger* MK981235, especially after the powder of the carb shells was involved as bioadsorbents (Abdel Wahab *et al.* 2023). Byproducts resulting from chitinous waste fermentation were observed to enhance the dye-removal potential of *Paenibacillus mucilaginosus* TKU032, which exhibits strong adsorption capabilities. In case of adding fermented powder of shrimp heads as adsorbent, such capability of adsorption achieved 99% removal of Congo Red and 97% of Red No.7 (Doan *et al.* 2020). Similarly, using fermented squid pen powder, *Bacillus cereus* TKU034 achieved up to 99.5% adsorption of various disperse dyes (Liang *et al.* 2015).

Enhancement Strategies for the Production, Stability, and Activity of Microbial Chitinases

Recombinant expression of microbial chitinases

Chitinase synthesis by genetic engineering and their subsequent expression in various strains of microorganisms represents a promising approach to develop recombinant strains with enhanced overexpressed chitinases and desired functional properties (Yu *et al.* 2022). Therefore, the genes that are responsible for the synthesis of thermostable chitinase in specific microorganisms can be easily cloned and expressed in different hosts (Sarma *et al.* 2013). Furthermore, the enzymes exhibit thermostable properties, maintaining correct folding under harsh conditions. They also possess resistance to host cell proteases and, therefore, are not degraded by these proteases (Sarma *et al.* 2013).

The shared repertoire of chitinase families between bacteria and fungi is a powerful illustration of Horizontal Gene Transfer (HGT), a key non-sexual mechanism driving microbial evolution (Goughenour *et al*. 2021). Evidence from phylogenetic studies consistently demonstrates that specific fungal chitinase genes exhibit a closer evolutionary relationship to bacterial counterparts, strongly indicating a bacterial origin for these clades (Gonçalves *et al*. 2016). This genetic exchange provides a substantial adaptive benefit, allowing the recipient organism to effectively break down chitin, which is a crucial structural component in both fungal cell walls and insect exoskeletons (Zhang *et al*. 2025). The cross-kingdom transfer is hypothesized to occur through various mechanisms, including conjugation-like events, the activity of transposable elements, and the intimate physical proximity within shared ecological niches (Richards *et al*. 2011). Moreover, the rate of HGT is closely linked to the organism's ecology, with parasitic and saprotrophic fungi showing elevated gene acquisition due to their constant interaction with bacteria (Liu *et al*. 2025).

The expression of chitinase genes can be increased by using innovative methods of biotechnology, such as cloning and recombinant technologies. Thus, the production and the activities of the enzymes will be developed and increased. Furthermore, several studies have reported the industrial and agricultural applications of cloned and overexpressed microbial chitinases in heterologous hosts. *E. coli* BL21 (DE3) successfully expressed the Mtch509 chitinase gene from *Microbulbifer thermotolerans*, producing a recombinant enzyme with high stability under elevated temperatures and in the presence of high salt concentrations (5 M NaCl) (Lee *et al*. 2018).

Bacillus subtilis, recognized as GRAS, can synthesize and secrete recombinant proteins extracellularly, although its application is limited by the lack of suitable expression vectors (Heravi *et al*. 2015). Yeast systems, such as *Pichia pastoris*, are ideal for heterologous gene expression due to their ease of genetic manipulation; chitinase expressed in *P. pastoris* reached maximum activity at 50 °C, with activity decreasing to 80% at 60 °C (Kaczmarek *et al*. 2021).

Similarly, *Saccharomyces cerevisiae* can efficiently express chitinase genes while performing post-translational modifications; for instance, *Thermomyces lanuginosus* chitinase expressed in *S. cerevisiae* exhibited optimal activity at pH 6.5 and 60 °C (Prasad and Palanivelu 2012).

Table 6 summarizes the enhancement of activity of these cloned microbial chitinase genes in various hosts.

Table 6. Cloned Microbial Chitinases from Various Microorganisms for Enhanced Enzyme Stability and Activity

Source	Gene/Enzyme	Expression Host	Functional Outcomes of Overexpression	Reference
Bacillus subtilis	Bs-chi and Sm-Chi	*E. coli* BL21	The recombinant chitinase maintained a higher residual activity of 86% at 45 ºC.	Bouqellah *et al.* (2024)
Metschnikowia pulcherrima	MpChit35 and MpChit38	*Pichia pastoris*	The three recombinant chitinases showed an activity at about 45 ºC and pH 4.0-4.5. MpChit35 and MpChit38 maintained 50% of their initial activity at 35 and 55 °C and maintained up to 50% of their activities at pH 3.0 and 3.5.	Minguet-Lobato *et al.* (2024)
Aeromonas sp.	ChiZJ408	*E. coli* BL21	The recombinant chitinase showed a high activity at 50 ºC and pH values between 4.0 and 7.0 with a maximum activity at pH 6.	Yu *et al.* (2022)
Thermomyces lanuginosus	N/A	*Pichia pastoris*	The recombinant chitinase showed a maximum activity was 50 °C, however, at 60 °C, it showed catalytic activity exceeding 80% of the maximum activity	Kaczmarek *et al.* (2021)

Microbulbifer thermotolerans	Mtch509	*E. coli* BL21	The recombinant chitinase showed great stability under acidic conditions, elevated temperatures and high salt concentrations.	Lee *et al.* (2018)
Saccharothrix yanglingensis Hhs	Chi6769	*E. coli* BL21	The recombinant enzyme displayed optimum activity at 49 ºC and pH 7.	Lu *et al.* (2018)
Paenibacillus barengoltzii	PbChi70	*E. coli* BL21	The recombinant bacterium showed the maximal growth at pH 5.5, 55 ºC and higher activity towards colloidal chitin mainly $(GlcNAc)_2$.	Yang *et al.* (2016)
Pseudomonas sp.	PsChiC	*E. coli* BL21	The recombinant bacterium showed hydrolytic activity towards the chitin tetrameric derivative and trimeric derivative	Zhong *et al.* (2015)
Halobacterium salinarum	HschiA1	*E. coli* BL21	The recombinant chitinase displayed optimum catalytic activity at pH 7.3 and 40 °C and showed high stability over broad pH (6–8.5) and temperature (25–45 °C) ranges.	García-Fraga *et al.* (2014)
Thermomyces lanuginosus	N/B	*Saccharomyces cerevisiae*	The recombinant chitinase displayed optimum activity at pH 6.5 and at 60 ºC	Prasad and Palanivelu (2012)

Genetic engineering strategies and directed evolution

To improve the level of expression and the activity of chitinase, certain gene modifications are required. However, at higher pH and temperature conditions, the enzyme stability and selectivity on the substrate may be affected and negatively changed (Okongo *et al.* 2019). Different methods have been established to obtain modifications in chitinase genes, such as site-directed mutagenesis, directed evolution, and the selection of desired properties (Berini *et al.* 2018). The process begins under extreme conditions with the isolation of the microbial enzyme, followed by rational mutagenesis and site-directed mutagenesis. For further improvement of the enzyme traits, direct evolution is applied (Sarma *et al.* 2013).

Table 7. Recombinant Chitinases from Various Microorganisms against Phytopathogens and Pests

Source	Gene/Enzyme	Expression Host	Target Pathogen	Reference
Bacillus halodurans	BhChitA	*E. coli* BL21	*Botrytis cinerea*	Ezzine *et al.* (2024)
Bacillus subtilis	Bs-chi and Sm-Chi	*E. coli* BL21	*Candida albicans, Alternaria solani,* and *Rhizoctonia solani*	Bouqellah *et al.* (2024)
Streptomyces sampsonii	Sschi61	*E. coli* BL21	Pestalotiopsis trachicarpicola)	Wang *et al.* (2022)
Chromobacterium violaceum	Chromobacterium violaceum	*E. coli* BL21	*Fusarium oxysporum* and *F. guttiforme*	Sousa *et al.* (2019)
Xenorhabdus nematophila	Chi60 and Chi70	*E. coli*	*Helicoverpa armigera*	Liu *et al.*, (2019)
Thermomyces lanuginosus	Chit1	*P. pastoris*	*Eldana saccharina*	Okongo *et al.* (2019)
Streptomyces sampsonii	ChiKJ406136	*E. coli* BL21	*Cylindrocladium scoparium* , *Cryphonectria parasitica Neofusicoccum parvum, & Fusarium oxysporum*	Li *et al.* (2018)
Saccharothrix yanglingensis	Chi6769	*E. coli* BL21	*Valsa mali*	Lu *et al.* (2018)
Bacillus pumilis	(ChiS)	*B. subtilis*	*Rhizoctonia solani* and *Trichoderma harzianum*	Rostami *et al.* (2017)
Myceliopthora thermophila	MtChit	*P. pastoris*	*Fusarium oxysporum* & *Curvularia lunata*	Dua *et al.* (2017)
Paenibacillus elgii	PeChi68	*E. coli*	*Cladosporium* spp. and *Botrytis cinerea*	Kim *et al.* (2017)
Thermomyces lanuginosus	Chit2	*P. pastoris*	*Penicillium verrucosum* and *Aspergillus niger*	Zhang *et al.* (2015)
Serratia marcescens	ChiA and ChiB	*Bacillus thuringiensis*	*Galleria mellonella* and *Drosophila melanogaster*	Ozgen *et al.* (2013)
Trichoderma atroviride	Chi42	*E. coli*	*Rhizoctonia solani, Sclerotinia sclerotiorum,* & *Alternaria alternata*	Matroodi *et al.* (2013)

The directed evolution methods can be applied to improve the thermal resilience of chitinase synthesized by the fungus *Beauveria bassiana* and the chitinase gene (Bbchit1) from *Erwinia carotovora* by DNA shuffling and screening (Fan *et al.* 2007). After deleting a single nucleotide in the sequence of chitinase encoding in *E. coli*, site-directed mutagenesis was used, resulting in a recombinant strain that exhibited activity at 90 °C and a pH ranging from 6.0 to 7.5 (Oku and Ishikawa 2006). Furthermore, the enzymatic thermostability and catalytic activities are being enhanced after the chitinase gene (ChiD)

of *Serratia proteamaculans* was manipulated by site-directed mutagenesis (Madhuprakash *et al.* 2012). Several works were designed for enhancing the plant-protecting activities against plant pathogens and pests by heterologous and homologous overexpression of chitinase, Table 7.

Recombinant chitinases in biocontrol and transgenic plant development

Biocontrol materials, when added to protect plants from phytopathogens, introduce them to high temperatures over an extended period. This highlights the significant importance of recombinant thermostable chitinase (Alves *et al.* 2018). For example, incorporating the carbohydrate-binding module from *Serratia marcescens* into *Trichoderma atroviride* Chi42 created a modified chitinase with enhanced antifungal activity (Matroodi *et al.* 2013).

Table 8. Disease-Resistant Transgenic Plants by Incorporating Chitinase Genes

Source	Gene /Enzyme	Transgenic Plant	Overexpression-Induced Resistance	Reference
Trichoderma atroviridae	*chit42*	*Saccharum officinarum*	Group of sugarcane pathogenic fungi *Fusarium proliferatum*, *F. subglutinans* , *F. verticillioides*, and *Alternaria* sp.	Matroodi *et al.* 2(024)
Phomopsis liquidambaris	*CHI*	*Triticum aestivum*	Fusarium head blight disease causal agent *Fusarium graminearum*	Zhu *et al.* (2022)
E. coli	*CsChi23*	*Cucumis sativus*	Fusarium wilt disease, causal agent (*Fusarium oxysporum)*	Bartholomew *et al.* (2022)
Coniothyrium minitans	*CmCH1*	*Glycine max*	Stem rot disease causal agent (*Sclerotinia sclerotiorum)*	Yang *et al.* (2020)
Trichoderma harzianum	*Chit42*	*Ipomoea batatas*	White rot disease causal agent (*Sclerotinia sclerotiorum)*	Ojaghian *et al.* (2020)
Trichoderma harzianum	*Chi*	*Solanum tuberosum*	Fusarium wilt and early blight diseases (*Alternaria solani* and *Fusarium oxysporum)*	Fatima *et al.* 2019)
Serratia marcescens	*SmchiC*	*Nicotiana tabacum*	*Botrytis cinereal* & *Spodoptera frugiperda*	Navarro-González *et al.* (2019)
Trichoderma harzianum	*Chit42*	*Daucus carota*	White rot disease causal agent *Sclerotinia sclerotiorum*	Ojaghian *et al.* (2018)
Trichoderma asperellum	*Chi (Tachi)*	*Glycine max*	Stem rot disease causal agent *Sclerotinia sclerotiorum*	Zhang *et al.* (2016)
Trichoderma atroviride	*Chit33*	*Brassica napus*	*Sclerotinia sclerotiorum*	Solgi *et al.* (2015)

Moreover, *E. coli* BL21 expressed the BhChitA chitinase gene of *Bacillus halodurans* and the recombinant necrosis-suppressing enzymes produced by *Botrytis cinerea* on tomato leaves (Ezzine *et al.* 2024). Similarly, the Sschi61 chitinase gene, synthesized by *Streptomyces sampsonii,* was successfully expressed by *E. coli* BL2,

whereas the recombinant chitinase inhibited the black spot pathogens of *Pestalotiopsis trachicarpicola* (Wang *et al.* 2022).

Incorporation of chitinase genes expressed in bacteria and fungi could successfully help in the production of pathogen-resistant transgenic plants using the above-mentioned techniques. The most popular method for this is plant transformation using *Agrobacterium tumefaciens* as a vector of chitinase genes. Chitinase genes from *Trichoderma* species are extensively employed to develop transgenic plants with enhanced pathogen resistance (Table 8).

A subsequent study reported that the expression of recombinant chitinase (CHI) from *Phomopsis liquidambaris* in *Glycine max* conferred transgenic plant resistance against head blight disease caused by *Fusarium graminearum* (Zhu *et al.* 2022).

Current Limitations and Challenges in Production and Application of Chitinases

Despite their promising potential, microbial chitinases face significant challenges in both research and industrial applications. The most prominent limitations include enzyme denaturation and instability under harsh processing conditions, which reduce their long-term effectiveness (Oyeleye and Normi 2018), Purifying chitinases from the fermentation broth typically involves multiple steps, which can be costly and may result in enzyme activity loss, thereby increasing the overall production expense (Singh *et al.* 2021). Recent studies suggest the potential of nanoparticles as effective inducers for improving the yield and catalytic efficiency of industrially relevant enzymes, including chitinases (Al-Rajhi *et al*. 2024). Additionally, the complexity of chitinase–substrate interactions and the need for precise reaction conditions impose constraints on scalability (Eijsink *et al.* 2008). The widespread use of chitinases, particularly in agriculture, raises concerns about their potential impact on non-target organisms that contain chitin, such as beneficial insects and fungi (Unuofin *et al.* 2024). This could lead to unintended ecological imbalances. while applications in transgenic plants raise ethical and regulatory concerns regarding environmental safety and consumer acceptance (Hasan *et al.* 2023).

CONCLUSIONS AND FUTURE PROSPECTS

1. Chitinases are valuable enzymes with broad applications in agriculture, biotechnology, medicine, and waste management. Future research is focused on expanding the functionality of chitinases, including their potential use as food preservatives, immunomodulators, and anti-tumor agents.
2. Advances in genetic engineering and enzyme modification are expected to enhance their stability, activity, and industrial viability. The development of thermostable chitinases through bacterial and fungal sources is a key priority, particularly for applications requiring prolonged enzyme efficiency under extreme conditions.
3. The biocontrol potential of chitinases in agriculture, as well as their medical applications in ophthalmic treatments and microbicides, highlights their diverse utility. Genetic engineering enhances chitinase stability and activity, increasing their industrial viability in extreme conditions.

4. As research progresses, the integration of biotechnology in optimizing chitinase production and function will be essential in making these enzymes more accessible and effective across various industries.

Data Availability Statement

Data is contained within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Use of Generative AI

During the preparation of this manuscript, the authors used ChatGPT to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

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