Protoplasma (2023) 260:1031–1046
https://doi.org/10.1007/s00709-023-01839-5

REVIEW

An overview of fungal chitinases and their potential applications

Deepali Thakur[1] · Aarti Bairwa[2] · Bhawna Dipta[2] · Prakriti Jhilta[1] · Anjali Chauhan[1]

Received: 29 May 2022 / Accepted: 30 January 2023 / Published online: 8 February 2023

Abstract

Chitin, the world's second most abundant biopolymer after cellulose, is composed of β-1,4-N-acetylglucosamine (GlcNAc) residues. It is the key structural component of many organisms, including crustaceans, mollusks, marine invertebrates, algae, fungi, insects, and nematodes. There has been a significant increase in the generation of chitinous waste from seafood businesses, resulting in a big amount of scrap. Although several organisms, such as plants, crustaceans, insects, nematodes, and animals, produce chitinases, microorganisms are promising candidates and a sustainable option that mediates chitin degradation. Fungi are the dominant group of chitinase producers among microorganisms. In fungi, chitinases are involved in morphogenesis, cell division, autolysis, chitin acquisition for nutritional purposes, and mycoparasitism. Many efficient chitinolytic fungi with potential applications have been identified in a variety of environments, including soil, water, marine wastes, and plants. The current review highlights the key sources of chitinolytic fungi and the characterization of fungal chitinases. It also discusses the applications of fungal chitinases and the cloning of fungal chitinase genes.

Keywords Biocontrol agents · Chitooligosaccharides · Chitinous waste · Exochitinases · N-acetylglucosamine

Introduction

Annually, about 10^{12}–10^{14} tonnes of chitin are produced in the biosphere (Dhillon et al. 2013), with the shrimp and crab shell wastes being the main source of extraction. Chitin degradation is an important phase in the environmental nutrient cycle process. The majority of these chitinous wastes are processed at ocean dumps, incinerated, or landfilled, resulting in natural resource wastage, economic loss, and environmental pollution. Under high-temperature conditions, chemical conversion of chitinous wastes to chitin requires steps such as demineralization, deproteinization, and deacetylation using strong acids and bases such as NaOH and HCl (Hahn et al. 2022). These methods impact the intrinsic qualities of pure chitin by affecting polymer parameters such as molecular weight, viscosity, purity, and degree of acetylation (Ploydee and Chaiyanan 2014). Furthermore, the purification of chitin by chemical methods is dangerous, energy-intensive, costly, and damaging to human health and ecological systems (Kim and Park 2015). Enzymatic deproteination and microbial-mediated fermentation are two biological approaches for chitin extraction (Kaczmarek et al. 2019). Chitin breakdown is predominantly carried out by microbial chitinases in nature. Chitinase breaks down chitin into oligosaccharides and monosaccharides, which flow into the microbial loop and are absorbed by other species. Microbial chitinases are critical for maintaining a balance between a high amount of carbon and nitrogen trapped as insoluble chitin in the biomass (Poria et al. 2021). Because of their huge abundance, cheap availability of raw material for their cultivation, low cost, variability in catalytic activity, and higher stability, microorganisms are considered the preferable source of chitinases in nature above plants and animals (Thakur et al. 2022). Chitinolytic microorganisms thrive on chitin-rich substrates. Considering the relevance of chitinous biomass bioconversion and the role of chitinases in the development of value-added products, the current review focuses on the diversity of chitinolytic fungi and applications of chitinases in a range of industries such as food, medicines, waste management, and agriculture. The prospect of

Handling Editor: Sonia Malik

✉ Bhawna Dipta
bhawna.dipta@gmail.com

[1] Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173230, Himachal Pradesh, India

[2] ICAR-Central Potato Research Institute, Shimla 171001, Himachal Pradesh, India

boosting native catalytic efficacy with a recombinant biotechnological tool has also been addressed.

Chitin: substrate for chitinase production

In 1811, Henri Braconnot, who was the director of the Botanical Gardens at the Academy of Sciences in Nancy, France, discovered chitin as an alkaline-insoluble fraction from mushrooms after treating them with dilute warm alkali and named it fongine/fungine (Braconnot 1811). Antonie Odier obtained a comparable alkaline-insoluble fraction from insect cuticles by repeatedly treating them with hot hydroxide solutions in 1823 (Odier 1823). The alkaline-insoluble fraction was given the term chitin which means "tunic" or "envelope." In 1843, Jean Louis Lassaigne demonstrated the existence of nitrogen in chitin while studying the exoskeleton of the silkworm butterfly *Bombyx mori* (Lassaigne 1843). Glucosamine and acetic acid were recognized as structural components of chitin by Ledderhose (1876). Gilson (1894) confirmed glucosamine as the repeating unit of chitin. The final chemical composition of chitin was elucidated by Purchase and Braun (1946). Chitin is a nitrogenous polymer that is rigid, inelastic, and hydrophobic, making it insoluble in water and most organic solvents. Chitin is structurally similar to cellulose, but instead of a hydroxyl group at the C-2 position inside the glucose unit, it has an acetamide group (Das et al. 2012). Chitin is found in insects, nematodes, fungal cell walls, mollusks, marine diatoms, and crustacean shells (Merzendorfer and Zimoch 2003; Adams 2004; Furuhashi et al. 2009; Rahman and Halfar 2014; Casadidio et al. 2019). Figure 1 presents the different sources of chitin in nature.

Chitin can be found in three different crystallographic forms α, β, and γ (Rudall 1969). The organization of polypeptide chains in these three polymorphic forms differs. The dominant and more stable form is α-chitin which is composed of antiparallel chitin microfibrils with strong intermolecular hydrogen bonding, whereas the β form is composed of parallel chains with weaker intermolecular forces, resulting in a more flexible and less stable molecule (Gardner and Blackwell 1975). The α-chitin can be found in fungal cell walls (Hassainia et al. 2018), insect cuticles (Sajomsang and Gonil 2010), and crustacean shells (Ifuku et al. 2009). The extracellular spines of the euryhaline diatoms *Thalassiosira fluviatilis* and *Cyclotella cryptica* were shown to contain a highly crystalline form of β-chitin (Blackwell et al. 1967). The β-chitin was also extracted from vestimentiferan

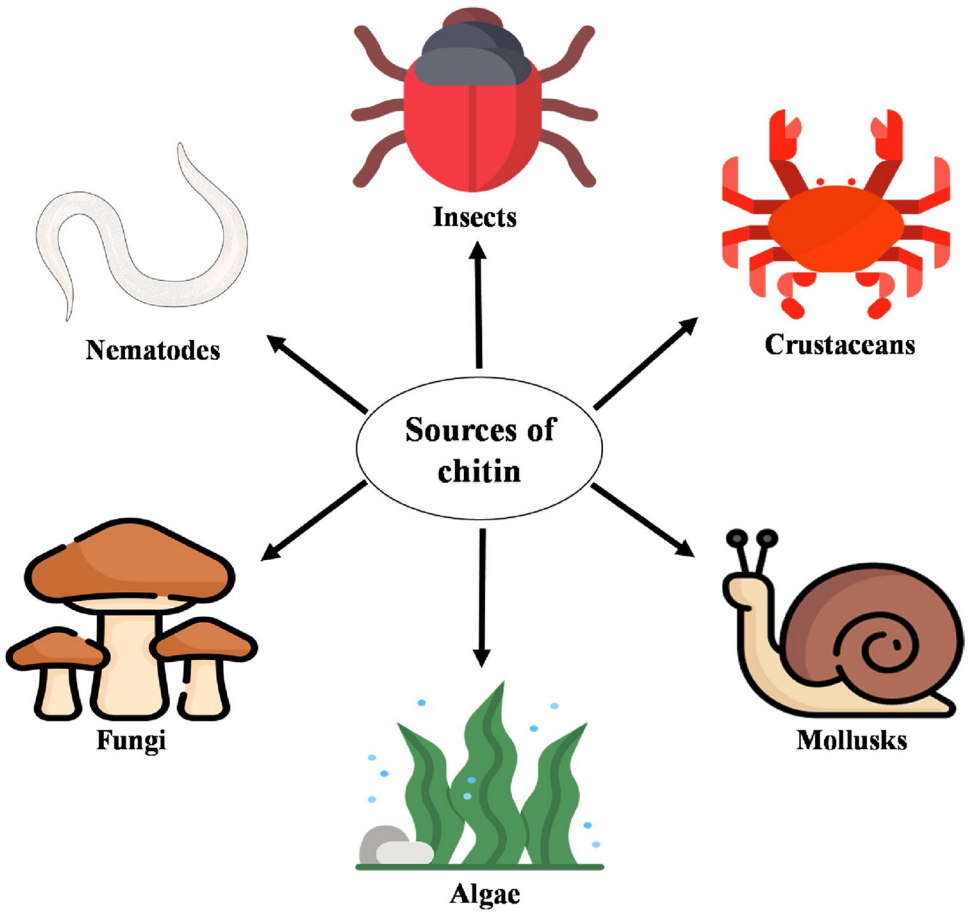

Fig. 1 Different sources of chitin in nature

Tevnia jerichonana (Gaill et al. 1992) and squid pens like *Todarodes pacifica* and *Loligo chenesis* (Youn et al. 2013; Cuong et al. 2016). The γ-chitin form has polymeric chains that are arbitrarily organized, with two parallel chains and one antiparallel chain forming the polymeric structure. The cocoon of the *Orgyia dubia* moth has γ crystallographic structure (Kaya et al. 2017).

Chitinases: a diverse category of enzymes that break down chitin

Bernard (1911) identified chitinase as a thermosensitive and diffusible antifungal component from orchid bulbs and later Karrer and Hofmann (1929) discovered this enzyme in snails. Chitinases (EC 3.2.1.14) are glycosyl hydrolases that may hydrolyze chitin into its oligomeric, dimeric, and monomeric components by cleaving the β-1,4 linkage between GlcNAc. Chitinase has two structural domains: the chitin-binding domain (CBD) for chitin attachment and the chitin-catalytic domain for chitin digestion (Arakane et al. 2003). CBD is essential for improving the efficacy of chitinases in degrading the ubiquitous biopolymer chitin or binding to a chitin-containing external surface (Frederiksen et al. 2013). Accessory domains such as fibronectin type III (FnIII) or cadherin-like domains link these two domains. Chitinases can be divided into two types based on their cleavage patterns: endochitinases and exochitinases. By randomly cleaving β-1,4-glycosidic bonds at internal locations in chitin, endochitinases (EC 3.2.1.14) produce soluble, low molecular weight oligomers. Exochitinases can be divided into two sub-categories. Chitobiosidases (EC 3.2.1.29) catalyze the progressive release of diacetylchitobiose from the non-reducing end of the chitin chain, whereas N-acetyl-glucosaminidases (EC 3.2.1.52) hydrolyzes the chitobiose to its monomeric unit, GlcNAc. Endochitinases are non-processive and have a shallow and open substrate-binding groove, whereas exochitinases are processive and tend to have a tunnel-shaped substrate-binding cleft (Horn et al. 2006; Sikorski et al. 2006). Chitinases mainly belong to four glycoside hydrolase (GH) families, i.e., GH18, GH19, GH23, and GH48. Family 18 chitinases use substrate-assisted catalysis to maintain the product's anomeric conformation, whereas family 19 chitinases use a general acid–base mechanism to invert the anomeric configuration of the hydrolyzed GlcNAc residue. The glycosidic linkages cleaved by GH48 chitinases use an inverting method of configuration. Chitinases from a wide range of species, including bacteria, fungi, archaea, mammals, plants, and insects, are found in Family 18 (Iseli et al. 1996; Gao et al. 2003; Arakane and Muthukrishnan 2010). Glutamate is used as a catalytic proton donor by chitinases in the GH23 family.

Chitin-binding proteins (CBPs) play a fundamental role in increasing the efficiency of chitinases in the degradation of the ubiquitous biopolymer chitin or to bind to an environmental surface containing chitin (Frederiksen et al. 2013). CBPs refer to a group of proteins that include one or more CBDs, but their binding affinity is not limited to chitin; it may also extend to a variety of complex glycoconjugates that contain GlcNAc or N-acetyl-D-neuraminic acid (NeuNAc) as building blocks (Raikhel et al. 1993). CBPs are categorized into three families CBM14, CBM18, and CBM33 of carbohydrate-binding modules. Families 14 and 18 CBPs are found in insects, fungi, and yeast, whereas family 33 CBPs are found mostly in bacteria (Vaaje-Kolstad et al. 2005, 2009).

Function of chitinases in diverse organisms

In nature, chitinases have a broad spectrum of distribution, including crustaceans (Wang et al. 2015), insects (Qu et al. 2021), nematodes (Dravid et al. 2015), plants (Ali et al. 2020), bacteria (Jenifer et al. 2021), actinobacteria (Brzezinska et al. 2019), fungi (Xie et al. 2021), archaea (Staufenberger et al. 2012; Hanazono et al. 2016), viruses (Rao et al. 2004; Wang et al. 2013), and animals (Hu et al. 2021). Chitinases play a variety of physiological and ecological roles in these organisms. Chitinases are involved in the degradation of the chitinous cuticle during molting in crustaceans, which is necessary for metamorphosis, development, and reproduction as well as digestion of chitin-containing foods and pathogen protection (Huang et al. 2010). During the molting process in insects, chitinases are shown to be involved in the digestion of chitin contained in cuticles and stomach lining (Muthukrishnan et al. 2020). In nematodes, chitinases are involved in several physiological processes, including egg hatching, larval molting, and reproduction (Chen et al. 2021). In plants, chitinases are part of the plant's defense mechanisms against pathogens (Karasuda et al. 2003). In bacteria, chitinases play an important role in parasitism, nutrition, and the recycling of chitin in nature (Itoh and Kimoto 2019). Human chitinases have been shown to protect against chitin-containing infections by degrading chitin found in pathogen cell walls (Kumar and Zhang 2019).

Chitinases are involved in different aspects of the life cycle of fungi, including (a) cell wall remodeling during spore germination and constriction, hyphal development, branching, and autolysis; (b) degradation of exogenous chitin present in the hyphal cell wall or the exoskeleton of arthropods for nutrition; and (c) competition with and defense against other fungi or arthropods in an aggressive pattern by killing the fungal prey first and then feeding on the dead cell contents (Seidl 2008; Wang et al. 2021).

Production of chitinases from fungi

In nature, fungi are the primary mediators of chitin degradation. Gooday et al. (1992) observed chitinase production in all stages of active growth of filamentous fungi, i.e., during spore germination, exponential growth, and mycelial development. Chitinases are involved in the autolysis associated with the release of spores from fruiting bodies of *Coprinus lagopus* (Iten and Matile 1970) and *Aspergillus nidulans* (Reyes et al. 1989). Chitinase also plays an essential role in cell separation during growth in *Saccharomyces cerevisiae* (Kuranda and Robbins 1991) and *Ustilago maydis* (Langner et al. 2015). GH18 chitinases are found in fungi; however, *Nosema bombycis* chitinase was the first GH19 chitinase discovered (Han et al. 2016). Fungal chitinases are classified phylogenetically into three groups: A, B, and C, each with its own domain architecture. Group A, the processive chitinases, contains a catalytic domain with a deep substrate binding site but no carbohydrate-binding modules (CBMs). They have a molecular mass of 40–50 kDa on average. Chitinases from group A are found in all fungal genomes. On the C-terminal of their catalytic domain, group B chitinases have a CBM or a serine/threonine-rich domain. Most non-processive chitinases belong to group B. The size, domain structure, and molecular weights of these chitinases differ. Small group B chitinases (30–45 kDa) that contain CBMs and large proteins (90 kDa) attached to the plasma membrane are two types of group B chitinases. Due to their deep substrate binding site, group C fungal chitinases are also processive, with a CBM 18 on the N-terminal of the catalytic domain. The presence of numerous lysine motifs (LysM) in fungal chitinase C, which are now classed as CBM family 50 in the carbohydrate active enzymes database (CAZy) database, is a distinguishing trait. The molecular mass of group C chitinases is typically 140–170 kDa. *Hypocrea atroviridis* (*Trichoderma atroviride*) Chi18-10 was the first chitinase described from group C (Seidl et al. 2005). Wang et al. (2021) speculated that group A chitinases play a major role in the growth and development of species, while group B chitinases are related to the mycoparasitic and entomopathogenic abilities of the fungi, and group C chitinases seem to be correlated with the host range broadening of some plant-pathogenic fungi in Sordariomycetes.

Fungal cell walls play a key role in maintaining mechanic stability during cell division and growth. Chitin is an important scaffolding substance found in fungal cell walls, where it offers mechanical stability (Brown et al. 2019). Polymeric chitin requires ongoing remodeling to preserve its plasticity, which is accomplished by chitinolytic enzymes. Chitinases can play a general role in cell wall plasticization or act more specifically during cell separation, nutritional chitin acquisition, or competitive interactions with other fungi (Langner and Gohre 2016). As the fungal cell wall is made of chitin, it has to protect itself from self-lysis. Gruber and Seidl-Seiboth (2012) hypothesized that the regulation of self and non-self fungal cell wall breakdown is not caused by chitinase speciation but is rather governed by substrate accessibility in healthy hyphae due to cell wall protection vs. deprotection during the mycoparasitic attack, hyphal aging, and autolysis. Fungi produce hydrophobic cell wall proteins like QID74 and carbohydrate-binding proteins to protect their cell walls from hydrolytic enzymes. QID74, a gene from *Trichoderma harzianum* CECT 2413, encodes a 74 kDa cell wall protein that plays a key function in mycelium protection in addition to its role in adhesion to hydrophobic surfaces (Rosado et al. 2007). Carbohydrate binding proteins bind to short oligosaccharides and chitin, preventing them from being degraded.

Trichoderma is one of the most extensively studied chitinolytic microorganisms. Diverse species of *Trichoderma* such as *Trichoderma harzianum* (De Marco et al. 2000; El-Katatny et al. 2001), *T. longibrachiatum* (Kovacs et al. 2004), *T. atroviride* (Harighi et al. 2007; Matroudi et al. 2008), *T. virens* (Ekundayo et al. 2016), *T. erinaceum* (Herath et al. 2015), *T. lixii* (Pasqualetti et al. 2019), and *T. asperellum* (Loc et al. 2020) are efficient chitin degraders. *Aspergillus* is also prominent chitinolytic fungal genera, including species such as *Aspergillus carneus* (Sherief et al. 1991), *A. terreus* (Krishnaveni and Ragunathan 2014; Farag et al. 2016), *A. niger*, *A. fumigatus* (Jenin et al. 2016), *A. griseoaurantiacus* (Shehata et al. 2018), *A. flavus* (Rawway et al. 2018), and *A. niveus* (Alves et al. 2018). Other chitinolytic fungi belong to the genera *Acremonium* (Gunaratna and Balasubramanian 1994), *Penicillium* (Rodriguez et al. 1995; Atalla et al. 2020), *Fusarium* (Mathivanan et al. 1998), *Alternaria* (Sharaf 2005), *Chaetomium* (Wang and Yang 2009), *Basidiobolous* (Mishra et al. 2012), *Paecilomyces* (Homthong et al. 2016), *Rhizopus* (Yanai et al. 1992; Sonawane et al. 2016), *Acremonium* (Chung et al. 2019), and *Clonostachys* (Pasqualetti et al. 2022). Several entomopathogenic fungi (EPF) such as *Nomuraea rileyi* (Wattanalai et al. 2004), *Isaria fumosorosea* (Ali et al. 2010), *Metarhizium anisopliae* (Staats et al. 2013), *Beauveria bassiana* (Svedese et al. 2013), and *Verticillium lecanii* (Yu et al. 2015) have been found to produce chitinases. Nematophagous fungi such as *Verticillium chlamydosporium*, *Verticillium suchlasporium* (Tikhonov et al. 2002), and *Monacrosporium thaumasium* (de Freitas Soares et al. 2015) have also been used as biopesticides against nematode eggs and larvae. The biochemical characteristics of fungal chitinases are depicted in Table 1.

Table 1 Biochemical characteristics of chitinases produced by fungi

Chitinolytic fungi	Activators	Inhibitors	Molecular weight (kDa)	Optimum pH	Optimum temperature (°C)	References
Candida albicans	-	-	-	5.0	50	Mellor et al. (1994)
Penicillium oxalicum	-	Hg^{2+} and Ag^{2+}	54.9	5.0	35	Rodriguez et al. (1995)
Piromyces communis OTS1	-	Hg^{2+} and allosamidin	44.9	6.0	50	Sakurada et al. (1997)
Myrothecium verrucaria	-	$ZnSO_4$, $CaCl_2$, and, Na_2EDTA	40	6.5	50	Govindsamy et al. (1998)
Trichoderma harzianum 1051	-	-	37	4.0	37	De Marco et al. (2000)
Aspergillus fumigatus YJ-407	-	Hg^{2+}, Pb^{2+}, Ag^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}	46	5.0	60	Xia et al. (2001)
Monascus purpureus CCRC31499	Fe^{2+}	Hg^{2+}	81	7.0	40	Wang et al. (2002)
Alternaria alternata	$CaCl_2$ and $FeSO_4$	$HgCl_2$, $CuCl_2$, and $PbCl_2$	-	5.0	30	Sharaf (2005)
Trichoderma atroviride PTCC5220	-	-	42	5.0	40	Harighi et al. (2007)
Penicillium sp. LYG 0704	Mg^{2+} and Mo^{2+}	Fe^{2+} and Hg^{2+}	47	5.0	40	Lee et al. (2009)
Isaria fumosorosea IF28.2	-	$CaCl_2$, $MgCl_2$, and $ZnSO_4$	-	5.7	25	Ali et al. (2010)
Aspergillus niger LOCK 62	-	Hg^{2+} and Pb^{2+}	43	6.0	40	Brzezinska and Jankiewicz (2012)
Gliocladium catenulatum strain HL-1–1	Co^{2+} and Ca^{2+}	Fe^{3+}, Cu^{2+}, and Ag^{+}	51	6.0	60	Ma et al. (2012)
Penicillium ochrochloron MTCC 517	-	Hg^{2+}, Zn^{2+}, K^{+}, and NH_4^{+}	64	7.0	40	Patil et al. (2013)
Verticillium lecanii	Mg^{2+}	Cu^{2+}, K^{+}, and Na^{+}	42	4.6	40	Yu et al. (2015)
Trichoderma viride	Ca^{2+}	EDTA and Mn^{2+}	-	5.0	50	Ekundayo et al. (2016)
Aspergillus terreus	Ca^{2+}, Mn^{2+}, and Na^{2+}	Hg^{2+}, Pb^{2+}, EDTA, ethanol, methanol, and acetone	60	5.6	50	Farag et al. (2016)
Aspergillus niveus	$MnCl_2$	KI, $CuSO_4$, and $ZnSO_4$	44	5.0	65	Alves et al. (2018)
Aspergillus flavus (AUMC 13576)	Mn^{2+} and Fe^{2+}	-	30	7.5	60	Beltagy et al. (2018)
Humicola grisea	Mn^{2+}, Co^{2+}, NH_4^{+}, and Mg^{2+}	Hg^{2+}, Ca^{2+}, Cu^{2+}, K^{+}, Fe^{2+}, Cr^{2+}, and EDTA	50	3.0	70	Kumar et al. (2018)
Penicillium chrysogenum MH745129	-	-	42	6.0	40	Atalla et al. (2020)
Trichoderma asperellum PQ34	Al^{3+}, Fe^{3+}, Fe^{2+}, and Ca^{2+}	Zn^{2+} and SDS	-	7.0	40	Loc et al. (2020)
Trichoderma bissettii strains GJ-Sp1 and TOP-Co8	-	-	-	5.0	50	Chung et al. (2022)
Thermomyces lanuginosus	β-mercaptoethanol	Cu^{2+}, Hg^{2+}, and EDTA	18	6.5	50	Suryawanshi and Eswari (2022)

Sources of chitinolytic fungi

Chitinolytic fungi have been reported to exist in a variety of environments, including shore soil, marine sediments, and seawater. Tweddell et al. (1994) isolated chitinolytic *Stachybotrys elegans* from the soil in Izmir, Turkey. *S. elegans* was shown to have strong antagonistic activity against *Rhizoctonia solani*. Patidar et al. (2005) isolated *Penicillium chrysogenum* (PPCS 1 and PPCS 2), *Aspergillus flavus* PAFS 3, and *Aspergillus niger* PANS 6 from the chitin-rich soils of shrimp drying fields. Isolate PPCS 1 yielded maximum chitinase (3809 U g^{-1} initial dry substrate) at pH 5.0, inoculum size 1×10^6 spore ml^{-1}, and 80% initial moisture content. Eight fungal species, viz. *Aspergillus flavus*, *A. niger*, *A. foetidus*, *A. ungius*, *Alternaria alternata*, *Cladosporium herbarum*, *Fusarium equisitum*, and *Dendryphiella vinosa* characterized by chitinolytic activity were isolated from Egyptian black sand collected from the Rosetta coast. *A. alternata* was the most promising species for chitinase production. The crude *A. alternata* chitinase showed 82% mortality of the larvae of the fruit fly (Sharaf 2005). Another chitinolytic fungus, *Lecanicillium psalliotae* was isolated from soil samples in Yunnan Province and deposited as the strain code CGMCC1312 in the China General Microbiological Culture Collection Center (Gan et al. 2007). *Paecilomyces javanicus,* an EPF of coleopteran and lepidopteran insects, was isolated from infected pupae of casuarina (*Lymantria xylina*) (Chen et al. 2007). Lee et al. (2009) procured chitinolytic fungi *Penicillium* sp. LYG 0704 from the soil of the crop field at Chonnam National University. The molecular mass of chitinase was estimated to be 47 kDa. *Plectosphaerella* sp. strain MF-1 was isolated from marine substrates such as calcareous shells, plant materials, and wood blocks. SDS-PAGE revealed that purified chitinase has a molecular mass of 67 kDa. Among the metal ions tested, Ag^+, Hg^{2+}, and Pb^{2+} strongly inhibited enzyme activity, whereas Mg^{2+} and Fe^{2+} had minimal inhibition. "DNISQTGEHARYXPMVWFIKL" was found to be the N-terminal amino acid sequence (Velmurugan et al. 2011). Krishnaveni and Ragunathan (2014) isolated *Aspergillus terreus* CBNRKR KF529976 from marine soils in Pichavaram, Tamil Nadu. The strain successfully biodegraded four different marine wastes, i.e., crab shell, snail shell, shrimp shell, and fish scales, resulting in the production of highly active chitinase. Chitinolytic fungal strains, viz. *Paecilomyces* sp., *Gongronella* sp., and *Fusarium* sp. were isolated from soil in Thailand. *Paecilomyces* sp. showed higher shrimp shell digestion ability than *Gongronella* sp. and *Fusarium* sp. (Homthong et al. 2016).

Jenin et al. (2016) isolated a total of 10 fungi (J1 to J10) from an infected *Artemia parthenogenetica* sample collected from saltpan of Puthalam, Kanyakumari District, Tamil Nadu. In this study, 5 fungi (J1, J3, J4, J5, and J8) showed chitinolytic activity, and 2 best strains (J1 and J5) were selected for further study. Fungi J1 and J5 were identified as *Aspergillus niger* and *A. fumigatus*, respectively. In another study, *Trichoderma harzianum*, *T. viride*, and *T. hamatum* were isolated from soil sampled from agricultural fields and the rhizosphere of plantation crops in the north Gujarat region of India. *T. viride* was found to be a more promising isolate for the production of chitinase and showed antagonistic activity against fungal phytopathogens such as *Aspergillus niger*, *Fusarium oxysporum*, and *Sclerotium rolfsii* (Khatri et al. 2017). Shehata et al. (2018) isolated *Aspergillus griseoaurantiacus* KX010988 from algae and decayed salty wood samples collected from Egypt's Port Said Governorate. The chitinase activity was enhanced by Mn^{2+} and Zn^{2+} ions, while Fe^{2+} and Cu^{2+} ions strongly inhibited the chitinase activity. Chitinase also showed antifungal activity against the pathogenic fungus *Fusarium solani*. Pasqualetti et al. (2019) screened twenty-eight fungal strains for the production of chitinolytic activity isolated from different natural marine substrates. The best chitinase producers were the halotolerant marine fungus *Trichoderma lixii* IG127 and the halophilic marine fungus *Clonostachys rosea* IG119. *Acremonium* sp. YS2-2, a chitinolytic marine-derived fungal strain, was isolated from seawater. The chitinolytic activity of the extracellular crude enzyme of YS2-2 was highest at pH 6.0–7.6 and a temperature of 23–45 °C (Chung et al. 2019). In another study, Atalla et al. (2020) isolated the chitinolytic marine fungus *Penicillium chrysogenum* MH745129 from red seawater. The optimum reaction mixture conditions for partially purified chitinase activity were obtained using 60% acetone at 40 °C, pH 6.0 at 40 min, and it was stable at 50 °C for 60 min. The molecular weight of chitinase was discovered to be 42 kDa. The linear mycelial development of both *Penicillium digitatum* and *P. italicum* was significantly reduced by chitinase. Twenty fungal strains were isolated from the tomato rhizosphere of Senegal. Among these 20 strains, *Trichoderma asperellum* TG4 showed the maximum chitinase activity at a temperature of 30 °C and a pH of 6.0 (Gueye et al. 2020).

Applications of fungal chitinases

The non-toxicity, biodegradability, biocompatibility, antimicrobial, and antiallergenic properties of chitin make it useful for a wide range of applications. The applications of fungal chitinases include biocontrol of phytopathogens and pests, generation of protoplasts, production of single cell proteins (SCPs), and production of chitooligosaccharides (COS) (Fig. 2).

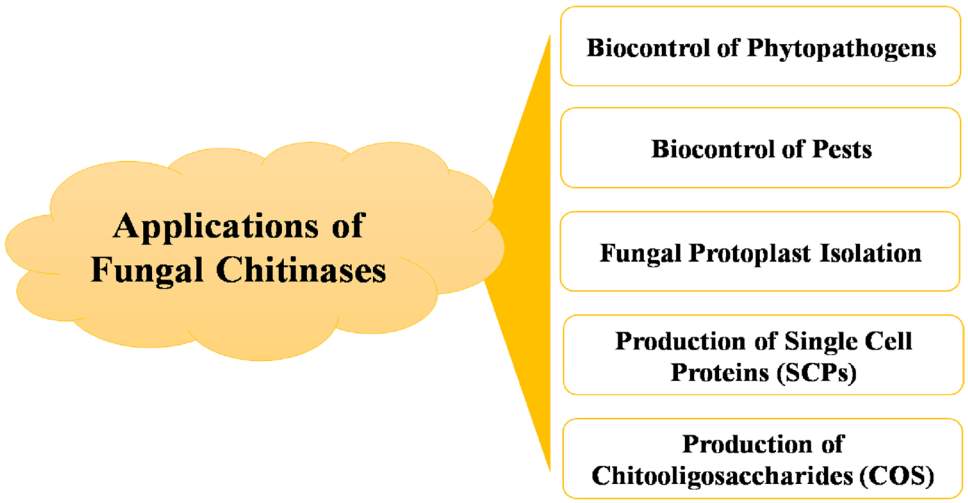

Fig. 2 Applications of fungal chitinases

Biocontrol of phytopathogens

Agricultural production has increased in recent decades, and farmers have become increasingly reliant on chemical fertilizers as a reliable form of crop protection approach. The increased use of chemical inputs, on the other hand, has presented a huge danger to food production and ecological stability around the world. Biological control is a potent and viable alternative to chemical control in agriculture. Mycolytic enzymes like chitinase, which operate as bioshields against phytopathogens, are produced by microbial communities. Chitinolytic fungi have been reported to affect fungal growth through the lysis of the cell wall (Tominaga and Tsujisaka 1976) and germ tube (Gunaratna and Balasubramanian 1994). Table 2 depicts the variety of chitinolytic fungi that have been reported as potential biocontrol agents against several plant pathogens.

Biocontrol of pests

Inappropriate use of pesticides can have detrimental repercussions for the environment and human health. There has been an upsurge in demand for biopesticides in agriculture due to the lower risk to human health and minimal detrimental impact on the environment (Kumar et al. 2021). Chitin is present in the cuticles of the epidermis, the trachea, and peritrophic matrices lining the gut epithelium of insects and the eggshell of nematodes. Fungal chitinases enzymatically split the chitin and cause the perforations that lead to illness and the death of the larvae. Elegant demonstrations of the involvement of chitinolytic fungi as an alternative to chemical pesticides are presented in Table 3.

Formation of protoplasts

Protoplasts are plasma membrane enclosed naked cells without a cell wall. The cell wall is removed mechanically or enzymatically to produce protoplasts. The formation of protoplasts from *Mulbranchea sulfurea* IMI 337,352 mycelia using chitinase and laminarinase produced by *Paecilomyces varioti* IMI 334,593 was reported by Gautam et al. (1996). They also reported a 34.6% frequency of protoplast regeneration. Using a chitinolytic enzyme produced by *Enterobacter* sp. NRG4, *Trichoderma reesei*, *Pleurotus florida*, *Agaricus bisporus*, and *Aspergillus niger* were reported to release protoplast (Dahiya et al. 2005). A purified 61 kDa chitinase from *Cellulosimicrobium cellulans* strain 191 was capable of protoplast formation from *Rhizopus oligosporus* and *Penicillium* sp. (Fleuri et al. 2009). With a 60% regeneration capacity, *Penicillium ochrochloron* MTCC 517 chitinase was extremely effective in the formation of protoplasts from *Aspergillus niger* (Patil et al. 2013). Sonawane et al. (2016) found that protoplasts of *Aspergillus niger*, *A. oryzae*, *Trichoderma viride*, and *Fusarium moniliforme* were efficiently produced using crude chitinase preparation from *Rhizopus stolonifer* NCIM 880. The protoplasts generated by *A. niger* and *T. viride*, respectively, showed 70 and 66% regeneration frequencies.

Production of single cell proteins

Production of single cell proteins (SCPs) from chitinous waste is a viable option for biomass production where chitinous waste is employed as a carbon and nutritional source. Fungal chitinase has the potential for producing SCPs, which might be used as a cheaper protein source. The bioconversion of chitin to SCP from shellfish waste by *Pichia*

Table 2 Chitinolytic fungi showing biocontrol activity

Target phytopathogens	Chitinolytic fungi	References
Puccinia arachidis	*Acremonium obclavatum*	Gunaratna and Balasubramanian (1994)
Sclerotium rolfsii	*Trichoderma* sp. isolate T_6	Lima et al. (1997)
Mucor plumbeus, *Cladosporium cladosporioides*, *Aspergillus versicolor*, and *Penicillium verrucosum*	*Verticillium lecanii*	Fenice et al. (1998)
Puccinia arachidis	*Fusarium chlamydosporum*	Mathivanan et al. (1998)
Crinipellis perniciosa	*Trichoderma harzianum* 1051	De Marco et al. (2000)
Sclerotium rolfsii	*Trichoderma harzianum* Rifai T24	El-Katatny et al. (2001)
Fusarium oxysporum and *F. solani*	*Monascus purpureus* CCRC31499	Wang et al. (2002)
Rhizoctonia solani	*Trichoderma atroviride* PTCC5220	Harighi et al. (2007)
Fusarium culmorum, *F. solani*, and *Rhizoctonia solani*	*Aspergillus niger* LOCK 62	Brzezinska and Jankiewicz (2012)
Alternaria alternata, Fusarium solani, Rhizoctonia solani, and *Aspergillus flavus*	*Basidiobolous ranarum*	Mishra et al. (2012)
Phytophthora meadii, *Rhizoctonia solani*, *Curvularia lunata*, *Corynespora cassiicola*, *Rigidoporus microporus*, *Fusarium oxysporum*, and *Colletotrichum gloeosporioides*	*Trichoderma erinaceum*	Herath et al. (2015)
Fusarium moniliforme, *Penicillium italicum*, and *Setosphaeria turcica*	*Verticillium lecanii*	Yu et al. (2015)
Aspergillus niger, *A. oryzae*, *Penicillum oxysporium*, *Rhizocotonia solani*, *Candida albicans*, *Fusarium solani*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*	*Aspergillus terreus*	Farag et al. (2016)
Aspergillus niger, *Fusarium oxysporum*, and *Sclerotium rolfsii*	*Trichoderma viride*	Khatri et al. (2017)
Fusarium solani	*Aspergillus griseoaurantiacus* KX010988	Shehata et al. (2018)
Aspergillus niger, *A. fumigatus*, *A. flavus*, *A. phoenicis*, and *Paecilomyces variotii*	*Aspergillus niveus*	Alves et al. (2018)
Colletotrichum sp. and *Sclerotium rolfsii*	*Trichoderma asperellum* PQ34	Loc et al. (2020)
Penicillium digitatum and *P. italicum*	*Penicillium chrysogenum* MH745129	Atalla et al. (2020)
Sclerotinia sclerotiorum and *Mucor circinelloides*	*Penicillium oxalicum* k10	Xie et al. (2021)
Trichoderma afroharzianum JRE 1A and *Aspergillus fumigatus* JRE 4B	*Fusarium oxysporum* and *Ganoderma boninense*	Mendrofa et al. (2021)
Aspergillus flavus and *Aspergillus niger*	*Trichoderma bissettii* strains GJ-Sp1 and TOP-Co8	Chung et al. (2022)
Curvularia lunata and *Fusarium oxysporum*	*Cladosporium cladosporioides*	Al Abboud et al. (2022)

Table 3 Chitinolytic fungi showing biopesticide activity

Target pests	Chitinolytic fungi	References
Globodera pallida	*Verticillium suchlasporium*	Tikhonov et al. (2002)
Meloidogyne javanica	*Paecilomyces lilacinus* strain 251	Khan et al. (2004)
Helicoverpa armigera	*Trichoderma harzianum* TUBF927	Binod et al. (2007)
Meloidogyne incognita	*Lecanicillium antillanum* B-3	Nguyen et al. (2007)
Plutella xylostella	*Metarhizium anisopliae* isolate M408	Wu et al. (2010)
Panagrellus redivivus	*Monacrosporium thaumasium* (NF34)	de Freitas Soares et al. (2015)
Bombyx mori	*Trichoderma viride*	Berini et al. (2016)
Galleria mellonella, Spodoptera littoralis, and *Agrotis ipsilon*	*Aspergillus awamori* EM66	Awad et al. (2017)
Culex pipiens	*Penicillium chrysogenum*	Mansour et al. (2019)
Culex pipiens	*Cladosporium cladosporioides*	Al Abboud et al. (2022)

Table 4 Cloning of genes involved in chitinolytic activity

Gene	Chitinolytic fungi	Feature	References
ech-42	*Trichoderma harzianum* 1 M1206040	Expression of *ech-42* in *Escherichia coli* DH5α showed chitinase activity and resulted in lysis of *Botrytis cinerea* cell walls *in vitro*	Carsolio et al. (1994)
chi1	*Trichoderma harzianum* strain T 25–1	*Saccharomyces cerevisiae* strain W3124 harboring the *chi1*-expression plasmid pC1CH1 showed activity on colloidal hydrated chitin, MUF-$(GlcNAc)_3$, and MUF-$(GlcNAc)_2$ substrates. The purified recombinant enzyme had a molecular mass of 44 kDa, an isoelectric point of 6.3 with optimum activity at a pH of 7.0, and a temperature of 20 °C	Draborg et al. (1996)
chi36	*Trichoderma harzianum Rifai* TM	Transformants showed endochitinase activity of *chit36* in glucose-rich medium and inhibited the growth of *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *melonis*, and *Sclerotium rolfsii*	Viterbo et al. (2001)
chit1	*Metarhizium anisopliae*	Recombinant protein, CHIT42, expressed in *E. coli* was active against glycol chitin and synthetic N-acetylglucosamine (GlcNAc) dimer and tetramer substrates	Baratto et al. (2003)
PjChi-1	*Paecilomyces javanicus*	*E. coli* BL21 (DE3) inhibited the growth of *Sclerotium rolfsii*, *Colletotrichum gloeosporioides*, *Aspergillus nidulans*, and *Rhizoctonia solani*	Chen et al. (2007)
ech42	*Trichoderma atroviride*	A high endochitinase concentration, 186 mg/l with a specific enzyme activity of 14,128 U mg^{-1} was produced in *Pichia pastoris*	Perez-Martinez et al. (2007)
chi46	*Chaetomium globosum*	*P. pastoris* GS115 harboring the *chi46* gene showed two protein bands with a molecular mass of approximately 46 kDa in the SDS-PAGE gel. The activity of recombinant enzyme was optimum at a temperature of 45 °C, pH of 5.0, and 5 mmol l^{-1} of Cu^{2+}	Liu et al. (2008)
chi58	*Chaetomium cupreum*	*P. pastoris* GS115 harboring *chi58* gene produced an extracellular chitinase that had an activity of 39 U/ml protein	Wang and Yang (2009)
cht2	*Trichoderma virens* UKM1	With a molecular weight of 35 kDa, the recombinant *cht2* was successfully expressed in *P. pastoris*. At a pH of 6.0 and a temperature of 35 °C, the partially purified enzyme had a specific enzyme activity of 1.34 U/mg	Al-Rashed et al. (2010)
Tachit1 and *Ctchit1*	*Thermoascus aurantiacus* var. *levisporus* and *Chaetomium thermophilum*	*TaCHIT1* and *CtCHIT1* produced molecular weight of about 48.4 and 47.3 kDa, respectively in *P. pastoris*. For *TaCHIT1* and *CtCHIT1*, the enzymes' peak catalytic activity occurred at pH 8.0 and 50 °C and pH 5.5 and 60 °C, respectively	Li et al. (2010)
chi42	*Trichoderma asperellum* SH 16	*S. cerevisiae* INVSc1 produced a fusion protein of approximately 50 kDa	Loc et al. (2013)
ifu-chit2	*Isaria fumosorosea*	The 50 kDa recombinant protein was efficiently produced in *E. coli* BL21 (DE3)	Meng et al. (2015)
chit46	*Trichoderma harzianum* GIM 3.442	Expression of *chit46* in *P. pastoris* GS 115 showed highest activity towards $(GlcNAc)_4$, $(GlcNAc)_5$, colloidal chitin and $(GlcNAc)_6$. Recombinant protein also effectively inhibited the growth of the phytopathogen fungus *B. cinerea*	Deng et al. (2019)
ChiB	*Aspergillus niger*	The recombinant strain *Bacillus subtilis* harboring pDG1663-*An*ChiB produced 3.7 mM of GlcNAc from 10 g of mycelial waste in 94 h with a yield of 71.3%	Liu et al. (2020)

Table 4 (continued)

Gene	Chitinolytic fungi	Feature	References
*Δ30Af*ChiJ	*Aspergillus fumigatus* df673	*E. coli* BL21 (DE3) produced chitinase with a molecular weight of approx 35 kDa and hydrolytic activity of colloidal chitin was found to be best at pH 4 and 45 °C	He et al. (2022)

kudriuvzevii chitinolytic enzyme has been reported by Revah-Moiseev and Carroad (1981). Vyas and Deshpande (1991) reported chitin enzymatic hydrolysis with the culture filtrate of *Myrothecium verrucaria* NCIM 903. Further, they reported the utilization of chitin hydrolysate by *Saccharomyces cerevisiae* NCIM 3052 for the production of SCP. SCP was found to contain 9.5 g/l biomass with 61% of total protein and 3.1% of nucleic acids. In another study, Patil and Jadhav (2014) reported that the chitinolytic enzyme of *Penicillium ochrochloron* degraded chitin to GlcNAc which was then employed as a substrate for SCP production by *Yerrowia lipolytica* NCIM 3450. SCP contained 65% total protein and 2.9% nucleic acid content with a biomass of 9.4 g/l.

Chitooligosaccharides production

Chitooligosaccharides (COS) are produced by the degradation of either chitin or chitosan via physical hydrolysis, acid hydrolysis, and enzymatic hydrolysis. COS has been the center of attention in the pharmaceutical, food, and agriculture sectors, mainly because of its higher water solubility, low viscosity, low molecular weight, biocompatibility, biodegradability, and nontoxicity. COS interacts readily with various cell receptors in living organisms, resulting in anticancer (Luo et al. 2016), antibacterial (Choi et al. 2001), antifungal (Mei et al. 2015), anti-HIV-1 (Artan et al. 2010), antihypertensive (Hong et al. 1998), antihyperlipidemic (Jiang et al. 2018), and antiobesity effects (Choi et al. 2012). COS also acts as a therapeutic agent against oxidative stress (Qu and Han 2016), diabetes (Ju et al. 2010), and inflammation (Kunanusornchai et al. 2016). COS also acts as an excellent preservative in food and enhances its shelf life. The use of COS inhibited *Lactobacillus brevis* contamination in the brewing process of beer, acting as an excellent preservative (Zhao et al. 2016). COS is also reported to stimulate several defense responses in plants (Lan et al. 2016).

Molecular cloning and expression of chitinase genes

The use of recombinant DNA (rDNA) technology as a tool for the development of genetically engineered microbial strains with selected characteristics for enzyme production is essential. Recombinant chitinases have shown greater characteristics than the native enzymes, which can be employed in the fermentation industry. Chitinase genes from a variety of fungal strains have been cloned and produced in suitable hosts for a variety of purposes. A chitinase gene, *Lpchi1*, was cloned from *Lecanicillium psalliotae* in *Pichia pastoris* GS115. *P. pastoris* expressed a recombinant chitinase of 45 kDa with optimal activity at a pH of 7.0 and a temperature of 37.6 °C. The purified chitinase also degraded chitinous components of the eggs of *Meloidogyne incognita* (Gan et al. 2007). Ramli et al. (2011) cloned a gene encoding a cold-adapted chitinase gene (CHI II) from *Glaciozyma antarctica* PI12 in *P. pastoris* GS115. The strain showed a strong affinity for colloidal chitin and little effect on glycol chitosan. The molecular weights of the purified recombinant chitinase were approximately 39 and 50 kDa, respectively. The enzyme was stable between pH 3.0–4.5 and could maintain its activity at temperatures ranging from 5 to 25 °C, with an optimal pH of 4.0 and a temperature of 15 °C. Prasad and Palanivelu (2012) cloned a chitinase gene from *Thermomyces lanuginosus* ATCC 44,008 in *Saccharomyces cerevisiae* SEY 2101. The molecular mass of the recombinant chitinase was 42 kDa, with optimum activity at a pH of 6.5 and a temperature of 60 °C. The recombinant chitinase showed excellent thermostability, retaining more than 60% of enzyme activity after 6 h at 50 °C. The cloning of fungal chitinase genes and their expression in microbial strains are demonstrated in Table 4.

Conclusion

Since chitinous waste is produced in large quantities, its valorization is essential to prevent environmental pollution. In this context, chitinolytic enzymes are critical toolboxes for chitin waste management and the generation of value-added products. Several organisms, including microorganisms, crustaceans, plants, insects, and animals, produce chitinases that mediate chitin degradation. Chitinases have a wide range of applications; hence, finding potent chitinases is critical for dealing with the growing problem of marine trash loaded with chitin compounds. Continued research into chitin breakdown will necessitate the development of novel enzymes with better activity and high-throughput chitinase tests. Further research is required to improve the market demand for chitinolytic enzymes.

Declarations

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

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