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MICROBIAL CHITINASES: EMERGING TRENDS IN BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

Chitinase plays significant role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource. Chitinases contribute to the production of carbon and nitrogen in the ecosystem. Chitinases are split into two broad categories as endo-chitinases and exo-chitinases. Common sources for chitinase production are insects, plants, mammals, bacteria and fungi. Enzymatic hydrolysis of chitin produces great interest in biomedical, industrial and agricultural applications. Production of low cost chitinolytic enzymes is significant in chitinous waste recycling as it is useful for the environment protection. In this review, various types of chitinases found in different organisms and its biotechnological applications are discussed.

Keywords: Chitinases, endo-chitinases and exo-chitinases, biocontrol

INTRODUCTION

Chitin

Chitin is a tough, white, inelastic linear polymer (Zikakis, 1984) and it is from the units of β -1, 4- N- acetyl glucosamine (GlcNAc). Chitin is the second most widely distributed biopolymer in nature (Singh *et al.*, 2014). It is found as the structural component of fungal cell walls, exoskeletons of arthropod, shells of cephalopods, cuticle of insects, shell fishes, squid and oyster. Enzyme chitin synthase is involved in the synthesis of chitin from activated precursor uridine diphosphate N-acetyl-D glucosamine (Glaser and Brown, 1957). X ray diffraction studies showed that depending upon the source, chitin occurs in three allomorphs, namely: α -, β -, and γ -forms (Younes and Rinaudo, 2015; Felse and Panda, 2000) and varies in the positioning of chains in the crystalline regions: α -chitin has an antiparallel chain arrangement (Minke and Blackwell, 1978), β -chitin has a parallel chain with intrasheet hydrogen bonding (Jang *et al.*, 2004), and γ -chitin, a hybrid of α -and β -chitin, has parallel and antiparallel orientations (Jang *et al.*, 2004). The most widespread and stable type is α -chitin, which is typically derived from exoskeletons of crustaceans, such as shrimps and crabs, β -chitin from squid pens, and γ -chitin from fungi and yeast (Filho *et al.*, 2007). The majority of expected applications are in the food industry, biomedicine, pharmacy, agriculture, tissue engineering, vaccine adjuvant, anti-tumour activity (Elieh and Hamblin, 2016). Chitin is a potential energy source as well as gene (Erbacher *et al.*, 1998) and drug delivery carrier (Dev *et al.*, 2010), and in the emerging field of nanobiotechnology (Koev *et al.*, 2006). Mostly 80,000 metric tons of chitin was taken from marine waste per year (Subasinghe, 1995; Muzzarelli, 1977).

Chitinases

Chitinases are widespread in nature and play significant role in the hydrolysis of \sim -I,4 linkages of the N-acetylglucosamine (GlcNAc) polymer chitin, an amino-polysaccharide present in different kinds of organisms mainly arthropods and fungi (Cabib 1987). Chitinases are glycosyl hydrolases, size ranging from 20 kDa to about 90 kDa (Bhattacharya *et al.*, 2007). Chitinase enzyme possesses ability of hydrolyzing insoluble chitin to its oligo and monomeric components (Cody *et al.*, 1990; Duo-Chuan, 2006; Goodday, 1990). Chitinases are the most important constituents of many bacterial species of which the best-known genera include *Serratia*, *Aeromonas*, *Vibrio*, *Bacillus* and *Streptomyces* (Cody, 1989). Enzyme chitinase has various applications such as production of pharmaceutically important chitoooligosaccharides and N-acetyl D-glucosamine (Usui *et al.*, 1990), production of single-cell protein (Revah-Moiseev and Carroad, 1981), in generation of protoplasts from fungi and yeast (Yabuki *et al.*, 1984), control of pathogenic fungi, chitinous waste management and transmission of malaria (Sakuda *et al.*, 1990; Sundheim *et al.*, 1988). Chito-oligomers produced by enzymatic hydrolysis of chitin are broadly used in various fields like in biomedical, agricultural and biotechnological applications, such as antifungal, antibacterial, antihypertensive and as a food quality enhancer (Boller, 1986; Goodday, 1986; Ordentlich *et al.*, 1988). Chitinase was first observed by Bernard in 1911 when he isolated a thermostable and diffusible chitinolytic fraction from orchid pulp which is next supported by Karrer and Hoffman for the presence of chitinolytic enzymes in snail (Flach *et al.*, 1992).

Classification of Chitinases

Chitinases belongs to the glycosyl hydrolase family because randomly it hydrolyze glycosidic linkage in a chitin molecule (Stoykov *et al.*, 2015). Chitinases are broadly classified as endo- and exochitinases (Lee *et al.*, 2009). The activity of endochitinase is defined as the randomly cleavage at internal points in the chain of chitin (Robbins *et al.*, 1998), and it splits β -1, 4 glycosyl linkages randomly at internal sites of chitin producing low molecular weight oligomers of G1CNAC such as chitotetraose, chitotriose and chitobiose. The exochitinases splits β -1,4 glycosyl linkage in chitin starting at the non-reducing end of chitin (Zhou *et al.*, 2019). Further exochitinases are sub divided into two categories namely chitobiosidase or β -(1, 4)- N-acetylglucosamine units (Robbins *et al.*, 1998; Tronsmo and Harman, 1993). Chitobiosidase cleaves alternate β -1,4 glycosyl linkage in chitin from the non-reducing end producing chitobiose (Yan and Fong, 2015; Hamid *et al.*, 2013) whereas, β -(1,4)-N-acetyl glucosaminidase cleaves β -1,4 glycosyl linkage in chitin from non-reducing end producing G1CNAC (Das *et al.*, 2016). It is also capable of acting on the oligomeric products of endochitinase and chitobiosidase to produce monomer moiety and that is G1CNAC (Annamalai *et al.*, 2010; Gortari and Hours, 2008).

Structural Aspects of Chitinases

Based on similar amino acid sequences chitinases can be grouped into glycosyl hydrolase families (GH) 18 and 19 and 20 which are unrelated structurally (Table 1). The catalytic mechanism of chitinases family 18 involves substrate-assisted catalysis, which retains the anomeric configuration of the product (Cohen-Kupiec and Chet, 1998). They are present everywhere with an (alpha/beta) 8-barrel fold structure in the catalytic domain (Coulson, 1994). Family 19, glycosyl hydrolases, share a homologous catalytic domain. They consist mainly of alpha helices. Their catalytic mechanism is a general acid-base mechanism that inverts the anomeric configuration of the hydrolyzed GlcNAc residue (Cohen-Kupiec and Chet, 1998). Family 19 chitinases mostly reported in plants (Itoh *et al.*, 2002), while Streptomycetes and human (β -N-acetyl hexosaminidases) chitinases are included in family 20 (Dahiya *et al.*, 2006; Roopavathi and Vigneshwari, 2015; Arakane and Muthukrishnan, 2010). Both GH 18 and 19 chitinases own signal peptides, representing that the enzymes are secreted and functional outside the cells. Family 18 of glycosyl hydrolases include active chitinases and inactive chitinase-like proteins or chito-lectins, which lack endogenous chitin and have been found widely in mammals (Funkhouser and Aronson, 2007). Recently comparative genomic analysis to explain the evolutionary history of the GH18 multiprotein family from early eukaryotes to mammals revealed that the GH18 chitinase involved in an emerging interface of innate and adaptive

immunity during early vertebrate history (Funkhouser and Aronson, 2007). Chito-lectins remains inactive due to lack of some critical residues in their catalytic sites. According to the N-terminal sequence, inducers, signal peptide, isoelectric pH, chitinases are classified into five different classes. Class I chitinases are found in plants. Class II are restricted to bacteria, fungi and plants. Class III are not similar in sequence with class I and class II. Class IV are similar in properties with class I but are considerably smaller. Class V chitinases are involved in interactions between plants and microbes (Matsumoto, 2014).

Roles of Chitinases

Chitinases are involved in many physiological and bioconversion processes. It plays important nutritional and parasitic roles in bacteria whereas in in-vertebrates, protozoa and fungi they are involved in morphogenesis. In case of plants and vertebrates, chitinases are involved in the defense mechanisms (Table 2). Baculoviruses, are usually used in biological system for control of insect pests, and also produces chitinases for pathogenesis (Gooday, 1995). Chitotriosidase enzyme was also used as marker of Gaucher disease, lysosomal storage disorder (Aerts *et al.*, 1996). Chitinase enzyme also possesses activity in human serum which was recently described and suggested possible role in defense against fungal pathogens (Escott *et al.*, 1996; Aerts *et al.*, 1996; Patil *et al.*, 2000). Chitinases play an important structural role in some fungi and arthropods than source of energy or defense part (Stoykov *et al.*, 2015). In the present days, chitinases gained increased attention because of their significant role in biocontrol of fungal phytopathogens (Mathivanan *et al.*, 1998).

Sources Of Chitinases

Plant Chitinases

In general plant chitinases are usually present in tubers, stems, seeds and flowers. As they are tissue specific, their molecular weight ranges from 20,000 to 40,000 Daltons. Based on the amino acid sequences, plant chitinases are categorized into five classes. Class I, II and IV are included in family 19, while III and V class chitinase belongs to family 18 (Roopavathi and Vigneshwari, 2015). Plant chitinases are more often considered as pathogen related (PR) proteins, as it is an induction of chitinase coordinated with the induction of specific p-1,3-glucanases and other pathogenesis related (PR) proteins because they are produced in response to attack by the phytopathogens (Malik, 2019). They are induced by growth regulators or elicitors such as chitoooligosaccharides. The acidic PR proteins are induced by salicylic acid while basic PR proteins are induced by ethylene. Their activity is also induced in response to stresses like high salt concentration, cold, drought, wounding and sometimes also by heavy

metal salts (Roopavathi and Vigneshwari, 2015; Hamid *et al.*, 2015; Kasprzewska, 2003; Eilenberg *et al.*, 2006). These chitinases play vital role in physiological processes of plants like growth and development process. Garg and Gupta (2010) stated isolation and purification of chitinase from moth beans against fungal pathogen. Chitinases isolated and purified from various plants like *Vicia faba*, *Vigna mungo*, and *Glycine max* play important role in regulation of nodulation by signal molecules (Eilenberg *et al.*, 2006; Sharma and Hooda 2018; Wang *et al.*, 2012; Chang *et al.*, 2014; Goormachtig *et al.*, 1998).

Fungal Chitinases

Fungal chitinases plays a prominent role in morphogenesis, nutrition and fungal development process. Chitin is the structural component of fungal cell wall (Sahai and Manocha, 1993). The primary structure of family 18 fungal chitinases mainly consists of 5 domains: (i) catalytic domain, (ii) N- terminal signal peptide region, (iii) chitin-binding domain, (iv)serine/threonine rich-region, and (v) C-terminal extension region. Fungal chitinases are not well defined like bacterial and plant chitinases and identified based on their similarity to family 18 chitinases from bacteria or plants (Takaya *et al.*, 1998a). Chitinases plays significant role in physiological and biological activities which include nutritional, parasitic roles, morphogenetic and autolytic. For instance, disruption of chitinase gene (CTS1) in the yeast *Saccharomyces cerevisiae* led to failure of the cell clumping, whereas in yeast, chitosanase and chitinase influenced morphogenesis (Shimono *et al.*, 2002).

Insect Chitinases

In insects, chitinases have been described from *Bombyx mori* and *Manduca sexta*. These enzymes behave as degradative enzymes and production of enzyme is controlled by hormones during larvae transformation. During ecdysis, endochitinases degrade cuticle into chitooligosaccharides. Further exochitinases hydrolyses chitooligosaccharides into Nacetyl-glucosamine which helps to synthesize a new cuticle (Hamid *et al.*, 2013; Koga *et al.*, 1997).

Mammalian Chitinase

Mammalian chitinases belong to family 18 of glycosyl hydrolases. In mammals, chitin is not found however, carbohydrates like hyaluronic acid and heparin sulfate are used as substrate in place of chitin (Lee *et al.*, 2011). Moreover 7 chitinases are identified in mammals which are further classified as true chitinases and chitinases like proteins (CLPs). Enzymatic activity to degrade chitin can be seen in true chitinases and CLPs do not show enzymatic activity (Roopavathi and Vigneshwari, 2015; Bussink *et al.*, 2007). Chitotrisidase and acidic mammalian chitinase (AMCase) are true chitinases which poses a catalytic and

chitin binding domain (Barad *et al.*, 2019). Chitotrisidase shows antifungal property and is produced in macrophages and expressed in Gaucher's cells while AMCase is produced by eosinophils, macrophages and epithelial cells (Lee *et al.*, 2011; Roopavathi and Vigneshwari, 2015). These enzymes when active shows specific pattern during different disorders and can be used as markers for detection and study of various inflammatory and malignant disorders (Kzhyshkowska *et al.*, 2007).

Microbial Chitinases

In the present days, the need of production of microbial chitinase has increased, and it serves for two purposes, reduce environmental hazards and increases production of industrially important value-added products. Chitinase production from microorganisms is higher when compared to other higher organisms (Matsumoto, 2014).

Bacterial Chitinases

Bacterial chitinases belongs to the category of family 18 of glycosyl hydrolases; however, *Streptomyces* species enzyme has been put under family 19. Chitin binding domain is present is present either at the N-terminal or C-terminal end of the enzyme (Hamid *et al.*, 2013; Morimoto *et al.*, 1997). Representing the amino acid sequence, bacterial chitinases are divided into three subfamilies A, B, and C (Watanabe *et al.*, 1999). Subfamily A chitinases have the presence of a third domain corresponding to the insertion of an $\alpha+\beta$ fold region between the seventh and eighth (α/β)₈ barrel (Dahiya *et al.*, 2006) 3, 14, 17 (Yan and Fong, 2015; Roopavathi and Vigneshwari, 2015; Hamid *et al.*, 2013). Research carried on characterization and purification shows variation in the bacterial chitinases, molecular weight ranging from 20 to 60kDa (Brzezinska *et al.*, 2014). Optimum pH varies from 5 to 8, while, optimum temperature ranges from 30° to 40° C (Dukariya and Kumar 2020; Xia *et al.*, 2011; Annamalai *et al.*, 2010). Majority of chitinases characterized from bacteria have been classified into subfamily A, suggesting they are vastly distributed in nature (Hamid *et al.*, 2013; Cohen-Kupiec, and Chet 1998). Bacteria mainly produces enzyme chitinase to obtain its nutritional requirement such as carbon and nitrogen and for parasitism (Bhattacharya *et al.*, 2007; Funkhouser and Aronson, 2007; Patil *et al.*, 2000; Júnior *et al.*, 2018). Among microorganisms, bacterial chitinases play crucial role in the chitin degradation process, to meet its energy demand (Hamid *et al.*, 2013; Cohen-Kupiec, and Chet 1998; Bhattacharya *et al.*, 2007; Patil *et al.*, 2000). Chitinase activity has been extensively found mainly in *Serratia*, *Streptomyces*, *Arthrobacter*, *Clostridium*, *Aeromonas*, *Klebsiella*, *Vibrio*, *Chromobacterium*, *Pseudomonas*, and *Bacillus* species (Stoykov *et al.*, 2015; Narayana and Vijayalakshmi, 2009; Brzezinska *et al.*, 2014). Heavy metals act as inhibitors of chitinase enzymatic activity (Donderski, and Brzezinska 2005).

Chitinases from Actinomycetes

Actinomycetes are well known producers of chitinases (Kumar and Singh, 2013; Mohanta, 2014). Researchers are exploring diverse habitats in an attempt to discover new actinomycete species for producing novel chitinase enzyme, having applications in various industries (Gurung *et al.*, 2013; Anbu *et al.*, 2015). Among rare *actinomycetes*, *Streptosporangium* sp, *Nocardiosis*, *Microbiospora* and *Micromonospora* have been reported as chitinase producers (Nawani, 2007; Shirlin, 2016). *Streptomyces*, the largest genus of Actinobacteria, mainly comprises of at least 500 species that are known as saprophytic bacteria which is an excellent source of chitinase suitable for the degradation of chitin (Christodoulou *et al.*, 2001). *Streptomyces* spp. degrades chitin with several chitinases that act synergistically (Seidl, 2008). *Streptomyces* has received particular attention for three main reasons. First, *streptomycetes* are abundant and important in the soil, where they play major roles in the cycling of carbon trapped in insoluble organic debris, particularly from plants and fungi. This action is enabled by the production of diverse hydrolytic exoenzymes. Second, the genus exhibits a fairly wide phylogenetic spread (Aderem, 2005). Third, among the nature's competent chemist, *Streptomyces* produce stunning bioactive secondary metabolites which is widely used in medicine and industry (Hopwood, 2007). They play a vital role in recycling of organic matter, in production of novel pharmaceuticals, enzymes, enzyme inhibitors, antitumor agents, immune modifiers and vitamins (Wellington *et al.*, 1993).

Production of Chitinases from *Streptomyces* spp

Streptomyces laurentii SN5 was isolated from the different sources such as shrimp shell waste and naturally died insects from the fish market of Jeddah. Colloidal chitin agar medium was used, the optimum pH value for production of chitinase was pH 7. Colonies with the luxuriant growth after incubating at 37°C for 3 days were considered as the best chitinase producers for bioconversion of disposable chitinous wastes. The enzyme obtained was purified using Sephadex G-100 and DEAE-Cellulose chromatography column and show similarity in molecular size of ~50 kDa. The isolate has enzyme activity of 0.533 (Ali *et al.*, 2020). The isolate *Streptomyces* sp. M1 from Mumbai sea water, possess an excellent chitinase activity with all types of substrates in short incubation period. The highest activity was observed in the presence of fungal chitin- 3.8 U/ml followed by insect chitin- 3.7 U/ml, shrimp chitin- 3.5 U/ml and crab chitin- 3.4 U/ml. *Streptomyces* sp. was capable of degrading chitinous substances (shrimp chitin, crab chitin, insect chitin, and fungal chitin) (Sukalkar *et al.*, 2017).

Streptomyces sp. ANU 6277 was isolated from the laterite soil and investigated for chitinase production for

submerged fermentation. 1% Chitin was used. Starch and yeast extract are good carbon and nitrogen source. Optimum temperature and pH for production was 35°C and 6 respectively. After purification by gel filtration, SDS PAGE displayed the molecular weight of chitinase as 45 kDa (Narayana and Vijayalakshmi, 2009).

Streptomyces mexicanus was isolated from Yamuna Bank, Delhi, Agricultural soil, Uttar Pradesh on yeast extract-malt extract agar plates. The culture was inoculated in 50 ml of colloidal chitin broth, incubated for seven days at 30°C and centrifuged to obtain cell free extract. Strain A showed 1.356 µmol/ml/min of enzyme activity, 0.225 mg/ml protein content in crude. SDS gel electrophoresis from the purified fraction mark the presence of single band of approximately 65 to 70 kDa. Analysis of purified chitinase were done by using MS/MS technique. N-terminal sequence corresponded to chitinase; the gene encodes a single protein of 453 amino acid residues (Das *et al.*, 2017).

Streptomyces sp. F-3, an extremophile thermophilic chitinase producer, was isolated from an alkaline-composting environment from Yucheng, Shandong, China, cultured at 50 °C in Luria–Bertani medium showed relatively high chitin degradation activities. Hydrolases expressed heterologously could resist to temperatures up to 70 °C and shows stability at pH values of 4 to 11 (Sun *et al.*, 2019).

Streptomyces tendae was isolated from the saline soil in Riyadh city. The optimum parameter for the production of chitinase was found to be temperature 35°C and pH 8.5 incubation time as 3 days. HPLC was done to detect the presence of amino acids in enzymes (Meena *et al.*, 2014). *Streptomyces* sp. MT7 was isolated from the loktak lake soil and it secretes three essential enzymes that undergo lysis of fungal cell wall-chitinase, β-1, 3- glucanase, and protease, and siderophores. Bio control traits like co-production of cell wall lytic enzymes and antifungal secondary metabolites including siderophores by *streptomyces* sp. suggests that it could be employed as a potential bio control agent against wood-rotting *basidiomycetes*. It also shows antifungal activity against white rot and brown-rot fungi *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Polystictus versicolor*, *Schizophyllum commune*, *Postia placenta*, *Gleophyllum trabeum*, *Polyporus friabilis*, and *Polyporus agaricus* (Nagpure *et al.*, 2013)

Industrial Usage of Chitinases

At present, chitinases have gained great attention in different biotechnological applications due to their chitin degrading ability from the cell wall of fungus and exoskeletons of insects allowing it to use as antimicrobial agent and insecticidal agent (Karasuda *et al.*, 2003; Mostafa *et al.*, 2009; Tsujibo *et al.*, 2003). Now-a-days interesting application of chitinase for recycling of

chitin, into pharmacological active products, namely N-acetylglucosamine and chito-oligosaccharides is in great use (Bhattacharya *et al.*, 2007; Dahiya *et al.*, 2006; Hayes *et al.*, 2008). Production of chito-oligomers through suitable enzymes is more appropriate for sustaining the environment rather than using chemical reactions (Songsiriritthigul *et al.*, 2009). Other importance gaining applications are the preparation of protoplasts from filamentous fungi, bio-control of insects and the production of single cell protein (Bhattacharya *et al.*, 2007; Hayes *et al.*, 2008). Therefore, chitinases have got great importance in biomedical and various biotechnological applications.

Table 1. List of organisms reported for chitinase activity

Organism	Functions	References
Bacteria	Degradation of chitin for energy utilization	Júnior <i>et al.</i> , 2018
Plants	Protection against plant pathogen	Júnior <i>et al.</i> , 2018 Renner and Specht, 2013
Fungus	Nutritional purposes, morphogenesis	Duo-Chuan, 2006; Adams, 2004
Insects	Cuticle degradation, defense against pests	Das <i>et al.</i> , 2016
Mammals	Defense mechanisms	Das <i>et al.</i> , 2016 Lee <i>et al.</i> , 2011

Fungal Protoplast Generation

Chitinases based on their property of degradation of chitin-containing cell wall is the acceleration of protoplast generation. This property helps to study the synthesis of cell wall, enzyme secretion, enzyme synthesis and fungal strain improvement by fusion of protoplast and development of new economically viable strains which may be further applied in various biotechnological industries (Yano *et al.*, 2006). Chitinase from *Burkholderia gladioli* CHB101 was identified with protoplast-forming activity against fungal mycelia (Shimosaka *et al.*, 2001). Chitinase expressed by *Streptomyces* was the most potent in generation of protoplasts from *Aspergillus oryzae* and *Fusarium solani* (Skujins *et al.*, 1965).

Single Cell Protein Production

Single cell proteins (SCPs) are usually used as a protein dietary supplement and can restore expensive conventional protein sources such as fish meal and soya meal (Nasseri *et al.*, 2011; Le and Yang, 2019). Chitin is mainly present in shell fish solid waste, which can be degraded by the enzyme chitinase to produce single cell protein (SCP) (Dahiya *et al.*, 2006). Wastes of shell fish is an abundant source of calcium carbonate, protein and chitin. Some of the fungal sources used for the production of SCP are *Saccharomyces cerevisiae*, *Candida tropicalis*, *Pichia kudriavzevii*, *Hansenula polymorpha*, and *Myrothecium*

verrucaria. The yield of single cell proteins was nearly 45% protein; 8-11% nucleic acids (Le and Yang, 2019). The production of chitinase enzyme from *S. cerevisiae* showed better results. Here the SCP produced was more than 60% and had lesser nucleic acid contents ranging from 1 to 3%. Evaluation of SCP production is based on total nucleic acid contents, protein amount and growth yield (Dahiya *et al.*, 2006; Roopavathi and Vigneshwari, 2015).

Production of Chitoooligosaccharides

The oligomers produced by chitin have vital role in biomedical and pharmaceutical fields. Second most copious biopolymer on earth, chitin is degraded by enzyme chitinase and forms various types of oligosaccharides like N-acetylglucosamine, chitoooligosaccharides, glucosamine and chitosan. The oligosaccharides which are formed act as elicitors in plant defense mechanisms, in biomedicine. It was reported that chitoooligosaccharides inhibited tumor growth indicating their antitumor function (Shen *et al.*, 2009) and they are also used in preservation of food items (Muzzarelli *et al.*, 2012),

Potential as Bio-Pesticides

In arthropods, insects and crustaceans are the major pests spread all over the ecosystems. Due to harmful effects and high cost of chemical pesticides, biopesticides being eco-friendly became popular due to their physiological role in nematode and insect (Herrera-Estrella and Chet, 1999). The gut lining and exoskeleton of insects is made up of chitin (Dahiya *et al.*, 2006; Okongo *et al.*, 2019) and insects have a chitinous cuticle, which can be degraded by chitinase (Roopavathi and Vigneshwari, 2015). Inhibitors of chitinases are found to be potent bio pesticides. Allosamidin which inhibits the growth of housefly larva and mite is a potent inhibitor of chitinases (Dahiya *et al.*, 2006). In transgenic plants, insect chitinases are used as biopesticides (Arakane and Muthukrishnan, 2010). *B. thuringiensis* transformed with heterologous chitinase genes and expands its use for pests and fungi control (Ramírez-Reyes *et al.*, 2004).

Biocontrol

Over the past 40 years, biomagnification has increased due the excessive use of fungicides which has led to the problems related to contamination and degradation of the natural environment, along with induced pathogen resistance. These harmful substances can destroy the beneficial insects and microbes in the soil, and can enter the food chain (Budi *et al.*, 2000). Biological control system, the use of microorganisms to control plant diseases, provides an alternative, environmentally sustainable approach to control plant pathogens. Recently, bio-control has given priority to microbes producing mycolytic enzymes, such as chitinases, which

Table2. Various chitinase producing streptomyces spp and their applications

Bacteria	Source of isolation	Application	References
<i>Streptomyces laurentii</i>	Shrimp cell waste	Environmental recycling of disposable chitin	Ali <i>et al.</i> , 2020
<i>Streptomyces coelicolor</i> ;	Soil samples	Bioconversion of lobster shells	Ilangumaran <i>et al.</i> , 2017
<i>Streptomyces albus</i>	Fish market soil	Waste management of chitinous wastes.	R. Santhi 2016.
<i>Streptomyces rubiginosus</i>	<i>Gossypium</i> rhizospheric soil	Bio-control of phytopathogenic fungi	Jha <i>et al.</i> , 2016
<i>Streptomyces anulatus</i>	Shrimp shells	Bio- control (Antifungal)	Aly <i>et al.</i> , 2010
<i>Streptomyces sp.</i>	Loktak Lake soil,	Bio control (secondary metabolites)	Nagpure <i>et al.</i> , 2013
<i>Streptomyces sp.</i>	Lonar Lake	Bio- control (Antifungal)	Bansode <i>et al.</i> , 2006
<i>Streptomyces mexicanus</i>	Agricultural soil,	Biodegradation of crustacean shells.	Das <i>et al.</i> , 2017
<i>Streptomyces rimosus</i>	Agricultural soil	Degradation of chitinous substances	Brzezinska <i>et al.</i> , 2012
<i>Streptomyces sp.</i>	Soil samples	Antibacterial	Deepika <i>et al.</i> , 2010.
<i>Streptomyces sp.</i>	Mongolian soil	Antifungal activity	Ja kim <i>et al.</i> , 2002.
<i>Streptomyces tendae</i>	Field soil	Antifungal activity	Abdulkhair <i>et al.</i> , 2012
<i>Streptomyces sp.</i>	Mangrove soil	Anticancer activity	Kalyani <i>et al.</i> , 2018
<i>Streptomyces gancidicus</i>	Agriculture soil	Antimicrobial activity,	Nayaka <i>et al.</i> , 2018
<i>Streptomyces sp.</i>	Palm Alajua soil	Antibacterial activity	Al_husnan <i>et al.</i> , 2016

Table3. Based on amino acid sequences classification of glycosyl hydrolases belonging to family 18, 19 and 20 (Beymoradi *et al.*, 2018; Lee *et al.*, 2011).

Glycosidase family	Classes	Occurrence	Structure
18	III and V	Bacteria, Actinomycetes, fungi, plants, insects and animals	Barrel folds
19	I, II and IV	<i>Streptomyces</i> and Plants	α - helixes
20	V and VI	Bacteria, <i>Streptomyces</i> and humans	Barrel folds

hydrolyze chitin, a main constituent of fungal cell wall. Chitinases or chitinolytic microbes holds promising as an alternative to chemicals due to their potential protection and lower environmental effects. Biological control system techniques have been an important approach for promoting sustainable agriculture (Hartl *et al.*, 2012; Kishore *et al.*, 2005; Prapagdee *et al.*, 2008; Sharma *et al.*, 2011). Chitinolytic bacteria also showed suppressing abilities for plant pathogens, such as *Paenibacillus* spp. and *Streptomyces* spp. against Fusarium wilt of cucumber (*Cucumis sativus*) caused by *Fusarium oxysporum*, *f. sp. Cucumerinum* (Stoykov *et al.*, 2015). The destruction of causative agent of witches broom disease of cocoa, *Crinipellis perniciosa* by using partially purified chitinase from *T. harzianum* was investigated (De Marco *et al.*, 2000).

Biomedical Applications

Chitinases can be used as promising additives in antifungal creams and lotions, as a bone-strengthenener

in osteoporosis, as antifungal agent (Ratanavaraporn *et al.*, 2009), as antibacterial agent (Rhoades *et al.*, 2006), as anti-malaria agent or a haemostatic agent in wound-dressings (Aam *et al.*, 2010), a vehicle for gene delivery (Koping-Hoggard *et al.*, 2004), and also in lowering effect on serum glucose level in diabetics (Lee *et al.*, 2003). The N-Acetyl glucosamine (GlcNAc) administered by oral routes, intramuscular (IM) and intravenous (IV) were identified as an inflammatory drug (Aloise *et al.*, 1996). Recently enzymatic hydrolysis of chitin producing chito-oligomers attained great attention due to their applications in biomedical fields like antihypertensive activity, immuno-enhancing effects, hypocholesterolemic and antitumor activity. Chitin is degradable by chitinases to generate chito-oligosaccharides such as chitohexaose and chitoheptaose, both of which have been reported to have anti-tumor activity (Patil *et al.*, 2000).

CONCLUSIONS

Chitinase producing microorganisms for a potential

biotechnological and pharmaceutical application may be produced in natural habitats. Apart from bioconversion of chitinous waste it has tremendous applications in production of chitinase enzyme which is used as biocontrol against various phytopathogens, in preparation of chito oligosaccharides, in generation of fungal protoplast, production of single cell protein, as bio-insecticide. Overall, it is also important to find alternatives to chemical pesticides as chitinases are important potent inhibitors for pests and pathogen control. However, this field of study is not yet sufficiently developed and although the findings so far are promising, more research in this direction should be pursued to obtain enough data to bring about an implied solution to the problems facing food production and harvest.

Future Perspectives

In the coming days, there is a great need for developing chitinase with various novel functions. Main focus is to improve its catalytic activity. Chitinases can be exploited in food technology by extending the shelf life of packaged foods. Novel therapeutic approaches can be made for several diseases such as chronic rhinosinusitis and asthma, by considering of the biological roles of various chitinases. Enzyme chitinases can be used to improve the human immune system. The chitin binding domain from the activity of chitinase can be swapped by applying protein engineering methodology. The above-mentioned approaches hold huge potential for the improvement of chitinolytic enzymes for future applications.

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