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Chitin and Chitinases, Production, Characterization and Applications

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Abstract: *Shrimp shells are used as substrates for the production of Chitinase by Actinomycetes and several species of bacteria used shrimp shells or colloidal chitin for the Chitinase production. Shrimp shell waste is used for culturing* of bacteria *on media for higher amounts of chitinolytic enzymes. The chitinolytic activity of bacteria of Streptomyces, Bacillus and Pseudomonas genera grown on shrimp and crab shell waste were recorded due to Chitinase production. Chitin can be biodegraded within 2 months in soils resulting in an upgrading in the numbers of soil microorganisms, a decrease of the harmful fugal flora (e.g., Fusuriurn) and preventing microbial infections of the plants which led to increases in plant production. This review aimed to clearfy the importace of chitin and chitinases in nature.*

Key words: *Chitin, Chitinases, enzyme, bacteria, applications*

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I. Chitin structure, presence and importance

The the most abundant naturally occurred polysaccharides after cellulose is chitin which is common in marine environments, fungi and insects. Approximately 75 % of the total weight of shellfish, such as shrimp, crab, and krill, is considered chitin wastes. Chitin is insoluble of unbranched linear chain of β-1, 4-linked N-acetyl D-glucosamine residues and chitin comprises 20–58 % of the dry weight of this waste which may find in combination with other polymers, such as proteins. In nature, two crystalline forms of chitin are found, the α-chitin form with antiparallel chitin microfibrils with strong intermolecular hydrogen bonding, the most abundant chitin in nature, found in shrimps and crabs and β-chitin form with parallel chitin chains and occurs in squid pens. Waste from the processing of marine crustaceans is a significant commercial source of chitin. Substantial amounts of chitin are produced in Asia, mainly India and Thailand, which are the major exporter of shrimp and utilization these wastes is rare, but if properly conducted, would solve the environmental problem of waste disposal and enable the great economic value of chitin to be utilized (Wang *et al.,* 2002a). The estimated annual amount of chitin produced by marine zooplankton is over several billions of tons (Boyer, 1994), and the total annual production of chitin is estimated to be 10^{10} to 10^{11} tons (Herwig *et al.,* 1988, Gooday, 1990, Tsugita, 1995). Surprisingly, the chitin content in marine sediment is quite low. This is due to bioconversion processes carried out by marine chitinolytic bacteria (Han *et al*, 2009), which transform this polysaccharide into organic compounds that are subsequently used by other microorganisms as a source of carbon and nitrogen. Common shrimp waste is processed for animal feed and is used in agriculture as a cheap natural nitrogen fertilizer. However, it is not uncommon for shrimp waste to travel from a drainage basin to stagnant waters, where different microorganisms decompose it. (Swiontek Brzezinska *et al*., 2008a).

Importance and uses of chitin:

Chitin has a broad range of applications, in food, chemical industries and medicine (Gooday, 1999, Patil *et al.,* 2000, Zhang *et al.,* 2000). Chitin and its related materials are also used in wastewater treatment (Flach *et al.,* 1992), as a traditional fertilizer in agricultural farming, drug delivery, and wound healing and as dietary fiber (Dixon, 1995, Muzzarelli *et al.,* 1999). β chitins are less stable and less abundant compared to α chitin because of the weak intermolecular or due to the parallel arrangements of chains (Sally Roopavathi, 2015).

Chitinases have many uses in human health care, in preparation of pharmaceutically important agents, preparation of single-cell protein, isolation of protoplasts from fungi and yeast, control of pathogenic fungi, treatment of chitinous waste and mosquito. Chitin and its associated materials have a broad usage in drug delivery, wound healing, dietary fiber, and in waste water treatment (Muzzarelli, 1999).

Chitinase enzymes:

Chitinases (E.C 3.2.2.14) belongs to glycosyl hydrolases, which hydrolyze the chitin to its monomer N-acetyl glucosamine by breaking the glycosidic bonds (Fukamizo, 2000). Chitinases molecular weight ranged from 20 - 90 kDa (Bhattachrya *et al.,* 2007). Chitinases have been divided into 2 main groups: Endochitinases and exochitinases. The endochitinases randomly split chitin at internal sites at random locations generating low molecular weight oligomers, such as chitototetriose, chitotriose, and diacetylochitobiose and forming the dimer di- cetylchitobiose and soluble low molecular mass multimers of GlcNAc (Sahai and Manocha, 1993). The exochitinases have been further divided into 2 subcategories: Chitobiosidases, (Harman *et al.,* 1993) which are involved in catalyzing the progressive release of di-acetylchitobiose starting at the non-reducing end of the chitin microfibril, and 1-4-β-glucosaminidases, cleaving the oligomeric products of endochitinases and chitobiosidases, thereby generating monomers of GlcNAc (Sahai and Manocha, 1993).

Chitinase are inducible (adaptive) enzymes, i.e., they are expressed only under certain conditions induced by a certain factor(s) and are regulated by a repressor/inducer system. While chitin is an inducer, glucose or another easily assimilable source of carbon may be a repressor (Sahai and Manocha, 1993). Frändberg and Schnürer (1994) reported the production of Chitinase, which was induced by chitin and chitooligosaccharides in the medium; however, no extracellular production of Chitinase was observed in the absence of these compounds. Numerous studies use colloidal chitin for the production of Chitinase (Mane and Deshmukh, 2009, Hosny *et al.,* 2010). However, some microorganisms can produce active Chitinase in the presence of shrimp shells (Wang *et al.,* 2002b, Chang *et al.,* 2010). Chitinase activity of *Trichoderma* indicate that a medium containing purified chitin or mycelium as the sole source of carbon provides optimum growth conditions for inducing extracellular Chitinase. Studies using different carbon substrates have confirmed the relationship between a metabolized source of carbon and the synthesis of chitinolytic enzymes. Chitinolytic activity was observed in bacteria cultured on media containing colloidal or the cell walls of certain fungi and minimal activity was observed in the same bacteria cultured on media containing glucose or laminarin as carbon source (Miyamoto *et al.,* 2007, Saks and Jankiewicz, 2010). Molecular studies have detected the presence of a two-component signal transduction system that regulates the synthesis of Chitinase in *Pseudoalteromonas piscicida* O-7, *Streptomyces thermoviolaceus* OPC-520, and other bacteria.

Characters of chitinase:

Microbial chitinase weights from 20 to 120 kDa, with most 20–60 kDa (Joo, 2005, Kavitha and Vijayalakshmi, 2011). The optimum pH and temperature of Chitinase are 5–8 and ~40°C, respectively. Depending on the origin of the chitinase, its activity can be inhibited or stabilized by the presence of various metal ions. A strong inhibitor of Chitinase is allosamidin, which was first reported as a specific, competitive inhibitor of insect chitinase. Allosamidin has a structure similar to that of an intermediate substrate, an oxazoline ring that may be formed between the carbonyl oxygen of the N-acetyl group and the C-1 of N-acetylglucosamine during hydrolysis (Koga *et al.,* 1987). 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 is restricted to bacteria, fungi and plants. Class III is not similar in sequence with class I and class II. Class IV is similar in properties with class I but it is considerably smaller.

The production of chitinase from microbial sources must take into consideration of optimized pH, temperature, inoculum size, specific suitable substrates such as chitin and suitable mode of fermentations (Meena *et al.,* 2014). *Serratia marcescens* QMB 1466 has a pH range of 4 to 7 and optimum temperature as 30ºC (Roberts and Cabin, 1982). Likewise, vibrio alginolyticus has a wide optimum pH range of 4 to 9 and optimum temperature for chitinase production is 40º C (Murao, *et al*., 1992). Because of the crystalline structure of chitin, it needs a thermostable chitinase enzyme to degrade the 2 million tonnes of shells of shrimp and prawns wastes per year in some countries. *Streptomyces thermoviolaceus*, *Bacillus* sp. BG 11 and Bacillus licheniformis X-7u are thermophilic microorganisms, which are major source for chitinase enzyme. Exochitinase which is thermostable was recovered from *Bacillus stearothermophilus* obtaied from organic solid waste compost (Haki and Rakshit, 2003). Chitinases are stable up to 40º C and pH ranges from weakly acidic to weakly basic. Water insoluble chitin could be used as a matrix for purification of chitinase by affinity chromatography (Stoykov and Pavlov, 2015).

Presence of Chitinase:

Chitinolytic microorganisms are capable of decomposing chitin under both aerobic and anaerobic conditions. They are found in many different environments and various organisms like viruses, plants, animals, fungi, bacteria, and insects. Contrary to the expectation that they abound in environments with high amounts of chitin, only a small number have been identified in shrimp waste: chitinolytic bacteria comprise only 4 % of the total heterotrophic bacteria. Chitinolytic fungi comprise 25–60 % of the total mold fungi, but their number is lower than the number of bacteria (Swiontek Brzezinska *et al.,* 2008a).

Fungal Chitinases:

Chitin is a major cell wall component of fungi (Sahai and Manocha, 1993). Fungal chitinases, like bacterial chitinases, have multiple functions as they play an important role in nutrition, morphogenesis, and fungal development processes. Fungal chitinases are not as well-classified as the bacterial and plant chitinases, and are identified on the basis of their similarity to family 18 chitinases from bacteria or plants (Takaya *et al.,* 1998a). It is thought that the fungal chitinases attach to their substrate or cell wall with the help of the chitin-binding domain. Moreover, the well-recognized mold fungus *Trichoderma harzianum* produces two N-acetylglucosaminidases, four endochitinases, and one chitobiosidase (Haran *et al.,* 1995). Chitin-degrading fungi include, for example *Aspergillus*, *Mucor*, and *Mortierella* (Schlegel and Jannasch, 2006). Similarly, *Trichoderma harzianum* and *Aspergillus niger* are also prospective chitinase-producing strains (Dahiya *et al.,* 2006).

Bacterial Chitinases:

The production of chitinases in bacteria is mainly for the degradation of chitin and many bacteria, including *Flavobacterium*, *Bacillus*, *Cytophaga, Pseudomonas, Serratia marcescens*, *Aeromonas* sp., and *S. griseus* HUT 6037 can synthesize several different Chitinase (Wang and Chang, 1997, Itoh *et al.,* 2002, Suzuki *et al.,* 2002). A number of bacteria have the ability to produce chitinases, including *Alteromonas* (Tsujibo, et al., 1993) *Escherchia,* (West and Colwell, 1984) *Aeromonas* (Sitrit et al., 1995). Chitinase-producing bacteria have been isolated from soil, shellfish waste, garden and park waste compost, and hot springs (Yuli et al., 2004). *Bacillus amyloliquefaciens* SM3 was isolated from the marine soil and exhibited high chitinolytic activity in colloidal chitin agar. Using optimized conditions the chitinolytic activity increased to three folds than unoptimized conditions (Das *et al.,* 2012). *Cellulosimicrobium cellulans* 191 was isolated from the alcoholic fermentation residues and checked for chitinolytic activity and the enzyme produced was purified and applied for formation of protoplast and lysis of fungi (Fleuri *et al.,* 2009). *Aeromonas schubertii* was isolated using colloidal chitin medium and enquired its chitinolytic activity. The extracellular enzyme was purified and characterized (Guo *et al.,* 2004). *Microbispora* sp. V2 was isolated from a hot spring at Vrajreshwari near Mumbai using colloidal chitin agar. Thermophilic and acidophilic chitinase produced was purified and characterized (Nawani *et al.,* 2002). *Bacillus licheniformis* strain was isolated from the mushroom bed and the novel thermostable chitinase (Waghmare and Ghosh, 2010).

Marine *Vibrio* (Keyhani *et al*., 2000), *Bacillus, Clostridium* (Konagaya *et al*., 2006) and *Micromonospora* (Nawani *et al*., 2002) can degrade chitin to chito-oligosaccharides, which can be metabolized and further used by others microorganisms as a sole source of nitrogen and carbon. Some other species of bacteria that also produce high levels of chitinolytic enzymes are *Serratia* (Watanabe *et al.,* 1997, Suzuki *et al.,* 2002). In aquatic environments, primarily heterotrophic bacteria decompose chitin. These include aerobic bacteria of the genera *Aeromonas*, *Enterobacter*, *Chromobacterium*, *Arthrobacter*, *Flavobacterium*, *Serratia*, *Bacillus*, *Erwinia*, *Vibrio* (Donderski and Trzebiatowska, 2000, Swiontek and Donderski, 2006). According to Swiontek Brzezinska *et al.* (2008b), 15 % of bacteria decomposed chitin in eutrophic Lake Chełmżyńskie. However, in the bottom sediments of this lake, a much lower number of chitinolytic microorganisms were identified. Mudryk (1991), in surface water of Lake Gardno, found that 10.6 % of bacteria degraded chitin, compared with only 5 % in sediment.

Mudryk, (1991) found chitinolytic bacteria in the feces of wild herbivores and domestic herbivores (e.g., sheep and cow). They were found in the rumen fluid of cows, which are unable to produce enzymes for digesting chitin and thus offer a living environment for chitinolytic bacteria. There is little information on the participation of anaerobic microorganisms in the degradation of chitin, although chitynolityc bacteria of the genus *Clostridium* have been described in marine environments (Boyer, 1994). *Cellulomonas* ATCC 21 399) is capable of rapid degradation of cellulose and chitin both aerobically and anaerobically. Similarly, Sturz and Robinson, (1985) observed that the degradation of chitin occurs mainly in surface sediments, where aerobic bacteria dominate and play a decisive role in its degradation.

Actinomycetes are a rich source of chitinase:

Actinobacteria from marine source are widely distributed in biotic sources such as fishes, molluscus, sponges, seaweeds, mangroves, in addition to seawater and sediments (Manivasagan *et al.,* 2014). Actinomycetes are rich sources of chitinase, which can be induced by the presence of chitin in the cultivation medium (Narayana and Vijayalakshmi, 2009). Swiontek Brzezinska *et al.,* (2013a) reported that 45–69 % of actinomycetes and 32–40 % of mold fungi were able to decompose chitin in the soil within the drainage basin of Lake Chełmżyńskie.

Chitinases production from genus *Streptomyces* sp.:

Paul and Clark, (2000) reported that ~90 % of all actinomycetes isolated from soil is of the genus *Streptomyces*. Swiontek Brzezinska *et al.,* (2008b) studied the decomposition of shrimp shells by *Streptomyces* sp. TH-11, they noticed a substantial reduction in the weight of the shells as early as the 7th, 12th, and 16th days. Marine *Streptomyces* sp. DA11 isolated from South China, found to be associated with sponge *Craniella australiensis* produced the enzyme chitinase and showed antifungal activities against *Aspergillus niger* and *Candida albicans* (Han *et al.,* 2009).

Streptomyces sp. ANU 6277 was isolated from the laterite soil was investigated for chitinase production for submerged fermentation (Narayana and Vijayalakshmi, 2009). *Streptomyces* sp. M-20 was isolated from the mangolian soil and the chitinase was purified and characterized. The molecular mass was exhibited 20 kDa by SDS PAGE (Kim *et al.,* 2003). Streptomyces sp isolated from the cerrado soil in Brazil were tested for its endo-chitinolytic activity and it showed antagonistic activity against phytopathogenic fungi (Gomes *et al.,* 2000*). Streptomyces aureofaciens* CMUAc130 is an endophytic actinomycetes isolated from the plant tissues like leaves, roots and stem of healthy plants. N -acetyl glucosamine alone does not induce the chitinase production but along with colloidal chitin increases the production of chitinase (Taechowisan *et al.,* 2003). *Streptomyces tendae* was isolated from the saline soil in Riyadh city. The optimum parameter for chitinase production was found to be temperature 35°C, pH 8.5 and incubation time of 3 days (Meena *et al.,* 2014).

Streptomyces sp. MT7 was isolated from the loktak lake soil and it secretes essential fungal cell wall lytic enzymes, chitinase, β-1, 3- glucanase, protease, and siderophores. It shows a broad range of activity against wood rotting fungi (Nagpure *et al.,* 2014). *Streptomyces lydicus* showed a broad range of antifungal activity (Mahadevan and Crawford, 1997). *Streptomyces halstedii*, *S. griseus*, and *S. cavourensis* SY224 produce highly active antifungal Genetic improvement of Streptomyces has been carried out by using mutation, conjugation, protoplast fusion, protoplast transformation, and recombinant DNA techniques for production of antibiotics and enzymes (Apichaisataienchote *et al.,* 2005). One mutant (SPVI) of *Streptomyces* sp. was found to overproduce an extracellular chitinase; an extracellular protein profile showed that the increased activity was due to increased synthesis of the enzyme protein (Vetrivel and Dharmalingam, 2000). Apichaisataienchote *et al.,* (2005) found that the recombinant strain SU-1 PFIS319 derived from *Streptomyces fradiae* SU-1 exhibited higher chitinase activity than the wild-type in chitinase induction medium. The parent secreted two chitinases with estimated molecular masses of 26 and 43 kDa whereas the recombinant strain secreted three chitinases of about 26, 31.5 and 43 kDa that inhibited the hyphal extension of *Fusarium moniliforme in vivo*. Synthesis of chitinase in *Streptomyces thermoviolaceus* is regulated by a two-component sensor-regulator system. For example, endochitinase from *Streptomyces violaceusniger* (Shekhar *et al.,* 2006) and thermostable chitinase from *Streptomyces thermoviolaceus* OPC-520 (Tsujibo *et al.,* 1993) have an optimum temperature of, respectively, 28°C and 80°C. In addition, the last enzyme has high pH optima in the range of 8.0 to 10.07, while the chitinase isolated from *Stenotrophomonas maltophilia* C3 has pH optima in the range of 4.5 to 5.0.

Induction of chitinase:

Like chitin, some of the other carbon sources for inducing chitinases production is chitobiose, chitooligosaccharides and glucosamine. It was reported that chitinase production was increased while using colloidal chitin. Chitin powder and chitin flakes poorly induce the chitinase activity. Compared to colloidal chitin, lactic acid processed chitin induces chitinase better (Stoykov and Pavlov, 2015). Extracellular chitinases are strongly affected by components of the media like carbon source, nitrogen source, presence of salts, residues from agriculture such as wheat bran, rice bran. When glucose was used along with chitin; catabolite repression was observed (Dahiya *et al.,* 2006).

Using of Chitinase:

Chitinases have the ability to degrade chitin directly to low molecular weight chitooligomers, which serve a broad range of industrial, agricultural, and medical functions such as elicitor action and anti-tumor activity (Yuli *et al.,* 2004). Chitinases have been receiving an increased attention due to their role in the biocontrol of fungal phytopathogens (Mathivanan et *al.,* 1998) and harmful insects (Mendonsa *et al.,* 1996). Chitinases have also attained a lot of attention as they are thought to play a key role in mosquito control and plant defense systems against chitin-containing pathogens (Mendonsa *et al.,* 1996).

Chitinase as antifungal agents:

Many authors have reported the isolation, purification, and characterization of chitinase from bacteria (Keyhani *et al.,* 2000; Konagaya *et al.,* 2006). Chitinases can be used as antifungal agents and have environmental and agricultural applications. Kishore *et al.,* (2005) reported that the lytic activity of chitinase on

the cell walls of pathogenic fungi can be correlated with the degree of biological control of these pathogens in vivo.

Dahiya *et al.,* (2006) believe that chitinolytic enzymes can be used as supplements for chemical fungicides to increase their effectiveness against pathogenic molds and reduce the required concentrations of these harmful chemicals. Microorganisms producing these enzymes can inhibit the growth of many fungal diseases that pose a serious threat to global crop production. Chitinases may be used to convert chitin-containing biomass into useful (depolymerized) components. Chitinases can be exploited for their use in control of fungal and insect pathogens of plants (Roberts and Selitrennikoff, 1988, Melchers and Stuiver, 2000). Fungal protoplasts have been exploited as a very efficient experimental means to study the synthesis of cell wall, enzyme synthesis, secretion, and strain improvement for biotechnological applications (Dahiya *et al.,* 2005a). Chitinase activity also acts as an indicator showing the activity of fungi in soil. It has been reported that there is a strong association between chitinase activity and fungal population in the soil. The chitinase effectively inhibit the growth of *R. solani, Marchophominia phaseolina* and *Fusarium* sp. (Nampoothiri *et al.,* 2004, Monteiro *et al.,* 2010). Moreover, Chitinase of bacterial origin were also found out to show fungicidal properties. For instance, Chitinase from *Bacillus thuringiensis* spp. *colmeri* inhibit growth of many phytopathogens, including *R. solani*, *B. cinerea*, *Penicillium chrysogenum*, and *Physalospora piricola* (Liu *et al.,* 2010). Prasanna *et al.,* (2013) stated that Chitinase from *Brevibacillus laterosporus* effectively inhibited development of *Fusarium equiseti*. These types of enzymes also showed insecticidal properties. Some yeast, e.g., *Pichia anomala* or *Pichia membranefaciens*, possess fungicidal properties as well. Their β-1.3-glucanases inhibited the growth of *B. cinerea* (Jijakli and Lepoivre, 1998, Masih and Paul, 2003). Development of this mold was also effectively inhibited by the chitinase produced by the following yeast: *Candida saitoana, C. guilliermondii*, and *C. oleophila* (El Ghaouth *et al.,* 1998, Saligkarias *et al.,* 2002).

Using of Chitinase inemoval of environmental wastes

Abilities of microorganisms to produce antifungal Chitinase are widely known.*Pseudomonas* sp. and *Bacillus* sp. effectively uses shrimp shells and wastes to produce chitinases. *Aspergillus* sp. produced more chitinases when it is grown in shrimp wastes compared to colloidal chitin medium. The chief components of solid waste from shellfish processing are $CaCO_3$, chitin, and protein. Chitinase from *S. marcescens* was used by their group to hydrolyze the chitinous material and yeast, *Pichia kudriavzevii*, in order to produce SCP that was acceptable as aquaculture. *Hensenula polymorpha, Candida tropicalis, S. cerevisiae*, and *M. verrucaria* have been commonly used for the production of SCP. The chitinase from *M. verrucaria* and *S. cerevisiae* have been used to produce SCP from chitinous waste by Wang and Hwang, (2001).

Medicinal importace of chitinase

Chitinases have a significant function in human health care. An important medical use for chitinases has also been recommended in augmenting the activity of anti-fungal drugs in therapy for fungal diseases (Orunsi and Trinci, 1985). Due to their topical applications, they have a prospective use in anti-fungal creams and lotions. Chitinases also have some other medical applications as well. For example, first discovery of the involvement of acidic mammalian chitinase in the pathogenesis of asthma was novel and unexpected because of the fact that mammals do not use chitin as an energy source, nor do they produce any chitinous structure (Zhu *et al.,* 2004). Several lines of evidence have demonstrated the importance of chitinases as an effector of host defense in the mammalian immune system. For example, humans that are deficient in chitotriosidase show an increased rate of microfilarial infection due to suppressed chitinolytic activity, allowing the parasite to thrive within the host.

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