

Chitinases Potential as Bio-Control

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ABSTRACT

The richest second polysaccharide in nature later than cellulose is chitin. It is present in the exoskeleton of yeast, fungi, algae and insects. It is also present in the inner structure of different vertebrates. The degradation of chitin is done by chitinases enzymes. In the environment nitrogen and carbon are generated by the contribution of chitinases. In biotechnological applications chitinolytic enzymes and chitin have gaining their significance. In the field of agriculture for the control of pathogens particularly the chitinases demoralized. Some human diseases particularly such as asthma, chitinases have a broad use for human health care. For the preparation of pharmaceutically significant chito-oligosaccharides and preparation of single cell protein, N-acetyl D glucosamine, separation of protoplast from yeast & fungi, for the handling of chitinous squander, to stop the pathogenic fungi and to stop the morphogenesis and mosquitoes etc chitinases has wider range applications. We are discussed a different type of chitinases and the chitinases present in a number of organisms for example plants, bacteria, mammals and fungi in this review.

Introduction

In the sixty's midst, of the emergent uprising, chemical pesticides consumption is considered while the best selection for pest's reserve. But continuously use of these pesticides brought a high quality of toxicity and had a negative effect on groundwater and soil throughout the world [1,2]. Persistent pesticides are those which strongly bind with soil particles and are immobile for a long period of time. To remove the chemical pesticides from soil and groundwater is very difficult. Due to continuous use, they decrease the crop yield, soil quality and hazardous effect on agriculture field [3,4]. Due to these harmful effects there is need to drive non-harmful alternatives. Biocontrol agents and enzymes are used for this purpose. Here we discuss chitinase enzyme which is used as a bio-pesticide [5]. Chitinase is an enzyme which belongs to hydrolytic enzymes which have the capacity to degrade the pathogens chitin e.g. insects and fungi and even the insect larvae [6]. The chitin is major cuticle component of membrane peritrophic which is the protective lining of the gut of many insects. The chitinase enzyme catalyzes this chitin into its monomers.

Many micro-organisms produce this enzyme in which fungi also include e.g. such as *Metarhiziumanisopliae*, bacteria name

ly *Bacillus pumilus*, *Serratia marcescens* and *Bacillus cereus*, actinomycetes such as *Streptomyces* spp. and yeasts such as *Tilletiopsiswashingtonensis* and *Tilletiopsisalbescens* [7-9]. Microbes which produce chitinase enzyme used for the control of insect nuisance and efficiency of bio-control related with production of chitinase. Polysaccharides happen in the numeral of crystalline form except α -chitin is recognized in the insect. Neighbor poly-GlcNAc chains work anti-parallel together in this form. Chitin includes different types of chitin-binding lectins which interact with that protein [10-12]. The mainly second rich biopolymer on this planet is chitin which is β -1, 4-N-acetylglucosamine (GlcNAc), is a linear polymer. It is present on the outside of the skeleton of fungi, algae, insects, shrimps, yeasts, lobsters and crabs. Chitin is also present in the inner structure of other invertebrates [13,14]. The dry weight of chitin is approximately 20-58% and 75% is the overall shellfish (such as shrimp, krill, and crab) weight which is likely as waste. Among a wide range of applications, the configuration of the extracellular chitinase is enhanced by the use of chitin [15,16].

The related materials of chitin have a wider use in the healing of wounds, delivery of drugs, treatment of wastewater and in di-

etary fiber. It is a chief part to make pollution in coastal areas and is the inelastic polysaccharide, hard and white. Chitin is a beneficial chelating agent because it contains (6.89%) nitrogen which is a high percentage. B-chitin and α -chitin are two allomorphic forms [17,18]. Polarities and packing of contiguous chains in the subsequent sheets are varying with these two forms of chitin. Chitinase degrades the chitin. Early cleavage of the polymer of chitin with the help of chitinases into oligosaccharides of chitin and additional cleavage to monosaccharides and N-acetylglucosamine by chitinase is a two-step process of chitin catabolism. 20kDa to 90kDa is approximately the size range of chitinases which are glycosyl hydrolases. These are present in broad varieties of organisms like yeasts, actinomycetes, bacteria, plants, arthropods, fungi and humans. Osteoarthritis treatment has been receiving particular concentration from N-acetylglucosamine (GlcNAc). The role in the bio-control of harmful insects and fungal phytopathogens chitinases has been getting better interest.

In the control of mosquito and defense system of plants beside chitin-containing pathogens chitinases play the significant role [19-22]. Human and animal diseases are caused by chitinases and chitin which are used by pathogens such as metazoan or protozoan. Exterior and interior (in host) environmental protection in pathogens is due to chitin coats. By the use of chitinase, they attack their host. By the use of host chitin-containing structures, the infection is successfully transmitted from one invertebrate to another. Different bacterial species such as *Escherichia*, *Streptomyces*, *Aeromonas*, *Alteromonas* have the ability to produce chitinase [23,24]. The bacteria which are producing chitinase has been remote from shellfish squander, compost of park waste, soil, burning springs and gardens [25]. There are two main groups of chitinases: Exo-chitinases and endochitinase. The chitin is split randomly at internal sites by endochitinases. Therefore, they form the di-cetylchitobiose dimer and dissolvable lower molecular mass of GlcNAc multimers while chitotetraose and chitotriose. There are two subcategories of exo-chitinases: The chitobiosidases are involving in catalyze the progressive discharge of di-acetylchitobiose which starts at the chitin microfibril non-reducing end [20,26-28]. 1-4- β -glucosaminidases cleave the oligomeric product of chitobiosidases & endo-chitinases, therefore, generate monomers of GlcNAc.

From this nomenclature for the strains HRO-C48, IC1270 and IC14 in *Serratia polymathic* have been approved by the recognition of varieties of chitinases. In the strains of HRO-C48 & IC 14 produces chitobiase or N-acetyl- β -1-4-D-hexosaminidase of 100kDa and endochitinase. 59kDa molecular mass of endochitinase, 50kDa of chitobiosidases and N-acetyl- β -D-glucosaminidases of 67 and 89 kDa are produced from the strains of IC1270. Chitinases amino acids similarity is considered from different organisms, there are five classes of chitinases have been projected and has been categorizing into two families. They contain 18 and 19 families of glycosyl hydrolases [12,25,28,29]. The large distribution in organisms, as well

as bacteria, plants, fungi (class 3rd and 5th), viruses and mammals, are from the 18 chitinases family. Single peptide, isoelectric pH, inducers, localization of the enzyme and N-terminal sequence are based by the chitinases subclassification. Plants are found in chitinases class I and enzymes which are present in fungi, plants and bacteria are in class II. Class I or II enzymes have no similarity in sequence in chitinases of class III. There are similarities in characteristics between class I chitinases and class IV chitinases which includes immunological property. Characteristics of class I chitinases have same as class IV, as well as immunological properties, other than they are considered minor than chitinases of class I [26,30]. Difference in molecular structure, catalytic mechanism and specificity of the substrate are shown by enzymes which are actually the chitinases diverse and huge group. It is beneficial to study the specificity of the substrate of chitinases, but it's not just revealed connection among physiological role and specificity of the substrate, it also allows ones to disgrace chitin into narrative foodstuffs which have industrialized application [5].

It is also known that all chitinase class can also exist in different specificity and reaction mechanism. In tobacco class 3 chitinase which consists of a large amount of lysozyme and also chitinase activity can also exist. In tobacco, class IV can also show the chitinase activity. In tobacco class iii and class, I can also use as an inverting mechanism which is used to the hydrolyzed beta-glycosidic linkage. While the class III chitinase in tobacco has a retaining mechanism. Two different families of chitinase cannot show or share the similar amino acid sequence and different 3D structures as well as molecular mechanism. These families can also evolve from different ancestors. Most of the plant have chitinase binding proteins can also exist which is in form of cysteine-rich chitin-binding domains as result of without the chitinase activity in plant cells. While in plant chitin binding protein are different from chitin binding protein of bacteria. In bacteria which contain 8 conserved cysteine domains. Tryptophan amino acid can also exist in chitinase domains of bacteria that are also involved in the binding of cellulose with cellulose [26,31].

Chitinase in Bacteria

In bacteria chitinase, chitin binding domain can also exist on the carboxy-terminal domains of enzyme and as well as amino terminal. Chitinase can be isolated and sequenced in most of the bacteria which contain family 18 glycosyl hydrolases. The catalytic site of other microbial chitinase and different form of chitinase is C-1. The amino-terminal of C-1chitinase shows similar characteristics and non-catalytic domain of other bacterial catalytic enzymes. Some other strain and species of bacteria can also produce the chitinolytic enzyme. Chitinase that is produced by bacteria its molecular weight is 20-60KDa that weight is smaller in weight of insect chitinase (40-85KDa) which are smaller in plant chitinase. Bacterial chitinase is active at high temperature and high Ph which also depend upon the chitinase that can isolate which type of source.

The different type of endochitinase has the wide range of temperature condition which in range 28-80C. Then pH of bacterial chitinase that is obtained in the range of pH 8-10.075 last time. A wide range of isoelectric points can show by bacterial chitinase. When we provide nutrient such as nitrogen and carbon as food supplement then it will produce the bacterial chitinase. The main point for the production of chitinase is that bacteria degraded the chitin and as result, it produces an energy source.

Some hydrolyze enzyme is present on the fungal cell wall that inhibits the fungal growth. Chitinase use as an antifungal agent that is helpful in biotechnology and use as food and seed preservative agents. Bacterial chitinase is characterized into 18 families. Then the most of bacterial chitinase exist in group A because they are most abundant in nature. Then group B and C are existing in nature are smaller quantity. Bacterial chitinase can exist in multiple functional domains such as CBD (chitinase binding domains) and Fn3 domains that are linked to the catalytic domain [8,9,31,32]. The degrading strength of CBD for soluble chitin has already been demonstrated in bacterial chitinase. It is also found that family 19 chitinase is only found in bacterial and plants cells. Multiple enzymes presence has been explained in various microorganism like *Bacillus circulans* WL-12, *Pseudomonas aeruginosa* K-187 and as *Aeromonas* spp. No. 10S-24. The synergistic function of chi-c1, chiA, and chiB of *S. marescens* 2170 has been published by Suzuki (2002) on the degradation of chitin. Instead of having similar catalytic domains Chi A and Chi B were consider as digesting chitin chains in opposite action i.e. Chi A from reducing end other from the non-reducing end [33].

Chitinase in Fungal

Like bacterial chitinase fungal chitinase has also multiple functions like in nutrition, the process involved in the fungal development and the morphogenesis. Chitin is major and important part of the fungal cell wall. There is a great similarity between the chitinase of fungi and class III of plant chitinase this homology is based on the amino acid. Mostly fungal chitinase are from family 18 of glycosyl hydrolase super family. In their structure, there are five domains these domains are as follows

- a) Catalytic domains
- b) N-terminal signal peptide domains
- c) Chitin binding region
- d) Threonine/serine-rich region
- e) C-terminal extension domain.

In most of the fungal chitinase the three, four and five number domains are absent. These domains are thought that they have no function for the chitinase action as it is observed that there are many enzymes which these regions and they do not has have still performed their enzymatic activity well. As bacterial chitinase is

well classified but the chitinase of fungi is not well classified and they are recognized based on the relation to the family 18 chitinases from bacteria or plants. So, the chitinase of fungi divided into plants or fungal chitinase which is related to class III chitinase and is identical to the class V chitinase from fungi, bacteria, and plants. Chitinase of fungi group C is not tilled characterized and is considered as a novel group of fungal chitinase. It is expected that they will be large as 140-170kDa and contain 2lysM domains and a chitin obligatory region. There is a great similarity between them and the toxins which kill the yeast. Its role as the physiological and biological activity is most important including autolytic, parasitic and nutritional roles.

For example, CTS1 chitinase gene disruption in the yeasts *S. cerevisiae* cause the cell unable to separate after the cell division and cause cell clumping this is explained in the (Lorito 1994), Ulhoa and peberdy 19191. They also try to explain in their research about cleansing and depiction of N-acetylglucosaminidase from different specimens of Trichoderms they also concluded by the Na-dodecylsulphate polyacrylamide gel electrophoresis that their molecular masses are similar. It is also reported that fungal chitinase involved in the filamentous sporulation as allosamidin and demethylallosamidin is inhibited by chitinase which led to the fragmentation of hyphae inhibition to arthroconidia. There are many chitinolytic fungi and bacteria that are used against a control of soil-borne pathogens of fungi. Chitinases from *Talaromyces flavus* after purification and characterization indicate their mycoparasitism of soil-based pathogens. (Harman 2007) and Yedidia explained that mycoparasitism is precious effects of Trichoderma on fungus it also includes resistance induction and increased development in plants. Yaun and Crawford 1995 reported that the antifungal biocontrol agent *Streptomyces lydicus* WYEC108 has the ability to destroy the fungal cell wall hyphae and damage developing oospores of some fungi. It is also concluded that different genes from the *Trichoderma harzianum* possess role in mycoparasitism. It is reported that ech42 gene disruption changes the extent of mycoparasitism in *T. harzianum*. The separation of a novel endochitinase called CHIT36 from *T. harzianum* explained by the viterbo 2001. More important application of fungal chitinase are

- i. Improving plant resistance with genetic manipulation
- ii. Anti-fungal activity against many phytopathogenic fungi
- iii. Insects control [34-36]

Chitinase of Plant

Chitinase is the structural unit in plants parts like the stems, seeds, flowers, and tubers. They are specific in tissues and regulated throughout development. Based on the sequence of amino acid plant chitinase is divided into six classes. The role of different domains is also involving in the classification. Out of six classes class I, II and IV enzymes contain globular domains. Class three and

four contains 8 alpha helices and 8 beta strands. In response to the pathogenic attack, plant chitinase produce pathogens related proteins as a self-defense there is some chitinase which is produce. Some of the chitinases which expressed themselves after environmental stress such as increased salt concentration cold and drought. The vital physiological process of some plants e.g. embryogenesis and ethylene synthesis also involve chitinase. Chitinase is made up of polypeptide chains of amino acids and acts as a major pathogenic protein which is accumulated in infected region e.g. tissue extracellularly [14,17,18,37]. In the study of Garg and Gupta, 2010 discuss purification and separation of chitinase enzymes whose sources are moth beans which can be used against fungal pathogens. *Macrophomina phaseolina* strain 2165. The plant's chitinase can be detected when plants are in their developing stages of growth the plant's chitinase is generally belong to the family of endochitinase whose weight is smaller than the weight of insect chitinase [24,38,39].

Insect Chitinases

The chitinases found within the insects are delineated from genus *Manduca sexta* and bombycid. These enzymes have vital roles to play as degradative enzymes throughout molting wherever endochitinases arbitrarily break the cuticle to chito-oligosaccharides that are a unit afterward hydrolyzed by exo-enzymes to N-acetyl-glucosamine. The chemical compound is reused for brand new cuticle synthesis. Insect chitinases conjointly play defensive roles against their own parasites, and therefore the accelerator production is regulated by hormones throughout the transformation of the larvae. All gramicidin is that the substance of insect chitinases. Chitinases also are found in crustaceans like shrimps, krills and prawns [22,40].

Mammalian Chitinases

The chitinase of mammals is a group of 18 of glycosylhydrolases (GH18), which divides into proteins that are similar to chitinase with 0% activity of enzymes. Chitotriosidase was an earlier mammalian chitinase to be recognized. The terminal catalytic domain of GH18 family members has fractions of triose-phosphate isomerase fold, that will be characterized by the (β/α) 8 - barrel structure, and in this barrel, β 4 strand contains conserved sequence motif. Glutamic acid is the basic portion that is giving a proton necessary for hydrolyzing the β (1-4) glycosidic bond present in chitin; this would be the re-arrangement of that compulsory glutamic acid to glutamine, leucine, and isoleucine that is responsible for the shortage of chitinolytic activity. Anyhow, the barrel will be neutral; it has now the ability of binding to chitin with huge affinity [3,4].

Methods of Production of Chitinase

"Fed-batch fermentation, continuous fermentation, and liquid batch fermentation", are the methods for the production of chitinase. $MgSO_4 \cdot 7H_2O$ and KH_2PO_4 have an excellent effect on the production of chitinase in the presence of chitin. On the other hand,

the fractions of yeast have the poor effect on chitinase production. The huge secretion of chitinase with lesser concentration levels is demonstrated by the components such as $MgSO_4 \cdot 7H_2O$, KH_2PO_4 , and yeast extract. The increasing effect of glucose on the production of chitinase is described by Bhushan, when glucose was utilized in the medium of production that contains chitin. However, Miyashita 1991, explain that glucose in the production of chitinase would be repressed. Some important factors such as pH, aeration, and temperature have great influence on the production of chitinase described by Bhushan 1998 [41-43]. To increase the chitinases production from several organisms, different methods, like biphasic cell systems, cell immobilization, solid-state fermentation are being used. Portions of both natural and general enzyme inhibitors are present, that has oxidizing/reducing agents. As recommended by crystallographic studies, a disordered Psammaplin A molecule reacts with the vicinity of the active site. Arkadin that is separated from *Clonostachys sp.* FO-7314 is one more inhibitor of chitinase. To attempt the cloning and expression of genes from different organisms into *E. coli*, various steps would be carried out with *S. plym disease*. The genes of Bacillus that encode ChiCW and ChiCH, have transformed into pGEX-6P-1 and show effectiveness in *E. coli*. Most bacteria of Streptomyces and non-Streptomyces have the great appearance in a production of chitin and also are antagonistic against *Sclerotinia minor* [44-46].

Uses

The useful purpose of chitinases is to change chitin-containing biomass into valuable (depolymerized) components. The control of insect and fungal pathogens of plants is also done by chitinase. The protoplast of fungal species will be explained in a very proper means of experiments to work and study the composition of the cell wall, secretion, enzyme synthesis, and strain improvement for applications of biotechnology. The activity of fungi in soil may also control by an indicator that would be enhanced from chitinase activity. The connection between chitinase activity and fungal population in the soil has been stated very strong. So, it seems that chitinases activity act like a proper indicator of the actively growing fungi in the soil [28, 47].

Medicinal Functions

Chito-oligosaccharides have an enormous pharmaceutical potential. The signaling for root nodule formation is done by them, where they behave as elicitors of defenders of plants and also have a potential to be used in human medicines. GlcNAc which is formed from glucose, then incorporated into glycoproteins and glycosaminoglycans in the human body is an anti-inflammatory drug [41,48-53]. The GlcNAc administered by oral routes, intravenous (IV), and intramuscular (IM) has been reported to be very energetic and very effective as an anti-inflammatory drug, that is used in the treatment of ulcerative colitis and other gastrointestinal inflammation disorders. Chitinases have an important function in human health care. Augmenting the activity of antifungal drugs in therapy for fungal

diseases has been assured by medical use of chitinase. They can have meaningful use in anti-fungal lotions and creams due to their typical applications [54-60]. The derivatives of chitin are also being used in the formation of artificial medical articles like contact lenses, artificial skin, and surgical stitches. Few of the derivatives of chitin are famously known to be non-allergic, nontoxic, biocompatible, and biodegradable due to the huge medical use of these kinds of compounds. Chitinases also have some other medical applications as well, such as the first invention of the involvement of acidic mammalian chitinase (AMCase) in the pathogenesis of asthma was unexpected and novel because mammals did not have the ability to use chitin as a source of energy, nor they generate any structure of chitinase [61-75].

a) Many pieces of evidence are represented regarding the importance of chitinases. It acts as associate degree effector in the class host system. As a bunch that lacks chitotriosidase is a lot of liable to infection. The explanation is that chitinolytic activity has become low. It permits the parasites to achieve success in the host.

b) Recombinant human chitotriosidase protein has the ability of anti-fungal activity. As a result of it will stop the assembly of *Candida albicans* hyphae formation [76-80]. Therefore, it conjointly helps in decreasing mortality in mouse models of neutropenic moniliasis and genus *Aspergillus*.

c) It absolutely was represented by the Zhu and colleagues within their 1st clinical findings that role of chitinase in bronchial asthma [respiratory disease | respiratory illness | respiratory disorder] wherever overstated quantity of AMCase were detected in the animal tissue cells and macrophages of respiratory organ biopsies taken from patients with asthma.

d) It absolutely was conjointly represented that the lungs of associate degree ovalbumin-induced mouse respiratory disease model may simply stimulate the AMCase level & BAL fluid chitinase activity.

e) It's conjointly found that the patients with the respiratory disease have the great quantity of chitinase-like proteins (e.g. YKL-40) in numerous respiratory organ tissues, liquid body substance and AMCase. Mostly mammals don't show the synthesis of polyose.

However, still, they need some chitinolytic enzymes.

- i. Acidic class chitinases: e.g. Chitotriosidase & AMCase
- ii. CLPs: chitinases like proteins
- iii. CBPs: breast regression proteins
- iv. BRP-39: Chondrocytes proteins

Chitotriosidase and AMCase typically show chitinase activity in mammals. There are 2 aspects regarding the activity of class chiti-

nases. 1st they carry chitin-binding domain (have half-dozen cysteine residues). So that they may have binding property. Secondly, CLPs don't have any binding sites. However contrary to the primary one it's conjointly high polyose binding affinity [81-90]. If amino alkanic acid is modified by essential amino acid in CHI3L1 then it couldn't ready to perform its chitinase activity. But stills its nice affinity for the chito-oligosaccharide and polyose. It's due to preserved substrate binding sites that weren't modified. It acts as the unhealthful go-between. With the assistance of chitinase containing pathogens, we will defend against the outside agents. Their proteins also are useful and show their effects in animal tissue cells of lungs, duct additionally as against macrophages. In case of human airway respiratory disease once due to TH2 cause inflammation, lungs are studied to point out unharness of AM cases. It absolutely was studied in allergic animal models. It absolutely was represented that just in case of thirteen effector pathway expression & TH2 inflammation, AMCases plays the essential role. In tissue inflammation and tissue modeling the supermolecule, CHI3L1 i.e. Chitinases 3-like super molecule one performs the crux role. It absolutely was studied in liver pathology, inflammatory disease, and cancer. Chitin, CaCO₃ and proteins area unit the most contents derived from the dead shell of shellfish. The chitinase gift in *Saccharomyces marcescens* is used to dissolve the polyose material of their own.

M. verrucaria, *S. cerevisiae*, *S. marcescens*, fungus tropicalis area unit the standard species that area unit accustomed get SCP. Wang and Hwang made SCP from polyose waste of *M. verrucaria* & *S. cerevisiae*. They conjointly represented that polyose chemical reaction might be performed through *verrucaria* chitinase and SCP may prepare from *S. cerevisiae*. Chitinases plays a serious role in agriculture and two-winged insects' management. It conjointly acts as A pesticides as AN additive [91-104]. It conjointly acts as fungicides. Thus, its production is incredibly useful in lowering the chemical sprays to avoid pollution. Chitin is additionally accustomed convert polyose waste into the fertilizers. Microorganisms secreting chitinase area unit wide get used. They're getting used to forestall plants pathogens and insects' pests to boosts the economy. Chitinase manufacturing organisms can even be used as biocontrol agents. Attributable to their generating proteins conjointly facilitate in dominant pests.

Future Prospects

Researchers are trying to seek out new functions of chitinase enzymes. Chitinase will is also accustomed increase lifetime of food as the food preservative. As we'd realize the enzymes and its therapeutic super molecule we have a tendency to treat the various diseases as like bronchial asthma and also the rhinosinusitis. Chitinase has the versions of chitohexose and chitoheptose that have anti-tumor activity. Thus, chitinase is also used because of the anti-tumor drug. It'd be accustomed enhance the human system. Researcher's area unit finding the new active sites of those enzymes. It'll lead towards the novel functions of chitinase enzymes.

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