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Chitinase: Production and applications

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ABSTRACT

Chitinases has an extensive range of applications currently in bio control, medical field, and degradation of pollutant, SCP production, bio pesticides and protoplast isolation. Chitinases are produced by diverse organisms like bacteria, fungi, plants, insects, humans and animals. Different processes like monoculture, co-culture, recombinant cells and immobilized cells are used for the enriched chitinase production. Recombinant strains and immobilized cells are used at the present time for enhanced production of chitinases. Here, we review the different sources, methods, and different factors responsible for chitinase production along with its applications.

Key words: Chitinases; Fermentation; Sources; Parameters; Application.

INTRODUCTION

Enzyme chitinases belongs to glycosyl hydrolases which hydrolyze the chitin to its monomer N-acetyl glucosamine by breaking the glycosidic bonds [12]. Chitin is a linear biopolymer which exists widely in fungal cell walls, arthropod exoskeletons, insect cuticles, shell fishes, mammals and plants. Chitinases are split into two broad categories as endo-chitinases and exo-chitinases [16]. Chitinases are produced in three different modes of fermentations - batch, fed batch and continuous fermentations. Solid state fermentations and submerged fermentations are used. Whole cell immobilization was also done for chitinase production. Co-culture is a promising technique followed to increase the chitinase production. It plays a wide role in parasitism, nutrition, morphogenesis, structural roles, and defence and energy source in bacteria, fungi and plants. Some of the commonly used sources for chitinase production are insects, plants, mammals, bacteria and fungi. Temperature, pH, incubation time and substrates have a major influence in the chitinase production. Chitinases are produced in a wide range of pH from 4 to 8 [39]. Optimum temperature range for chitinase varies from 30ºC to 50ºC. Chitin acts as an inducer in the medium for chitinase production or else chitinases are produced in low levels. Colloidal chitin, chitin flakes, cell walls of fungi, crab and shrimp shells are used as a carbon source. Some of the bioreactors used for chitinase production are stirred tank reactors, bubble column reactors, air lift reactor with draft tubes. Chitinase enzymes has a very broad of applications as biocontrol agent, morphogenesis, bioconversion of waste containing chitin, pollution degradation, mosquito control, fungal biomass estimation, protoplast isolation and bio pesticides. It is also used along with antifungal agents and also in skin lotions and creams for fungal infections. Due to its broad range of applications in agricultural and pollution degradation, there exists a strong interest to enhance the chitinase production for industrial purposes [9].

CHITIN

Chitin is a white, inelastic linear polymer and it is from the units of β-1, 4- N- acetyl glucosamine (GlcNAc). Chitin is the second most widely distributed biopolymer. It is found as the main component of fungal cell walls, cuticle of insects, internal shells of cephalopods, exoskeletons of arthropod, squid, shell fishes and oyster. Enzyme chitin synthase is involved in the synthesis of chitin from activated precursor uridine diphosphate N-acetyl-D-glucosamine.

X ray diffraction studies showed that it occurs in three different forms as α chitin & β chitin and γ chitin. Chains are arranged as anti-parallel and parallel monomers in α chitin and β chitin respectively. A chitin is compact and tight and forms crystalline structure.β chitins are less stable and less abundant compared to α chitin because of the weak intermolecular forces due to the parallel arrangements of chains [28]. Structural component of fungal cell wall and many invertebrates are made up of α chitin. Chitin also has a very broad range of applications in drug delivery, dietary fibres, waste water treatments and wound healing etc.75% of weights of the shell fish are considered as waste which contains almost 20% to 58% of chitin [16]. 80,000 metric tonnes of chitin were taken from marine waste per year. 20% to 40% of cell walls of fungi are made up of chitin [36].

CHITINASES

Bernard was the first person to observe chitinase [9]. Chitinases are glycosyl hydrolases which hydrolyze the chitins to it monomers by breaking the glycosidic bonds. Because of this nature, it is widely used in various biotechnological applications. Chitinases are divided into 2 broad categories: Endo-chitinases and Exo-chitinases. Chitinases are produced in all 3 different modes of fermentation *i.e.* batch, fed batch and continuous fermentation. Solid state fermentations are also used for the production of chitinases. Cell immobilization is the recent method used for the production of chitinase.

ROLES OF CHITINASES

Chitinases are present widely in various organisms like viruses, plants, animals, fungi, bacteria, insects and it plays a diverse role in these organisms. Chitinases are involved in many physiological and bioconversion processes. It plays a major role in nutrition and parasitism in bacteria. In case of plants and vertebrates, it is involved in the defence mechanisms. Chitinases are involved in morphogenesis in invertebrates, protozoa and fungi. Baculoviruses also produces chitinases for pathogenesis. Chitotriosodase enzyme was also used as marker of lysosomal storage disorder. Chitinase enzyme also has activity in human serum too [36]. Chitinases play a major structural role in some fungi and arthropods than source of energy or defence part[39].

CHITINASES CLASSIFICATION

Two broad categories of chitinases are endo-chitinases and exon-chitinases [16]. Endo-chitinases cleaves randomly at the internal sites of chitin and produces low molecular weight multimers such as chitotriose and chitotetraose. Exo-chitinases are further classified into 2 sub categories: chitobiosidases and 1-4-β glucosaminidases. The release of diacetylchitobiose which starts at the non-reducing end of chitin microfibril is catalysed by chitobiosidases. The oligomers obtained by endo-chitinases and chitobiosidases are cleaved by β-(1, 4) N-acetyl glucosaminidases.

Chitinolytic enzymes are grouped into glycosyl hydrolases families 18, 19 & 20 based on amino acid sequence similarity. Chitinases from family 18 and 19 use different mechanisms for hydrolysis. Family 18 use substrate assisted catalysis and family 19 use acid-base mechanisms [39].Bacteria, fungi, viruses, animals and plant chitinases are grouped under family 18. Plant chitinases and Streptomyces chitinases are grouped into family 19. Family 20 includes human and Streptomycetes. 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 [28].

SOURCES OF CHITINASES

Chitinases are produced by diverse microorganisms from different environmental conditions.

Bacillus amyloliquefaciens SM3 was isolated from the marine soil collected from different beaches in Tamilnadu and it exhibited high chitinolytic activity in colloidal chitin agar. Using optimized conditions the chitinolytic activity increased to three folds than unoptimized conditions [6]. *Cellulosimicrobium cellulans* 191 was isolated from the alcoholic fermentation residues and checked for chitinolytic activity and enzyme produced was purified and applied for formation of protoplast and lysis of fungi [11]. *Aeromonas schubertii was* isolated from soil of south part of Taiwan using colloidal chitin medium and enquired its chitinolytic activity. The extracellular enzyme was purified and characterized [14]. *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 [32].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* [17].*Bacillus licheniformis* strain was isolated from the mushroom bed and the novel thermostable chitinase produced by ion exchange chromatography and molecular weight was detected by SDS PAGE as 22 kDa. This important enzyme could be used in the bioconversion of waste containing chitin [46].

INSECT CHITINASES

The enzyme production in insects is regulated by hormones during the transformation of larvae[16].These enzymes act as degradative enzymes. Molecular weight of these chitinases varies from 40 to 80 kDa. Duringecdysis, the cuticle is degraded into chitooligosaccharides by endo-chitinases. Chitooligosaccharides are further hydrolysed to N-acetyl-glucosamine by exo-chitinases. This helps to synthesize a new cuticle. These chitinases plays a main role against their own parasite.

PLANT CHITINASES

Plant chitinases provides the ability of self defence against phytopathogens. According to the similarity in sequence, plant chitinases were divided into three classes and two additional classes have been found now. Class I chitinases consist of the sequence with a highly conserved main structure and an N-terminal Cys rich domain. Class II chitinases lack the Cys-rich domain but are structurally homologous to the main structure of class I chitinases. Class I, II, and IV enzymes can be included in family 19. Class III and V chitinases share no sequence homology with class I, II and IV enzymes, and belong to family 18, which includes most chitinases from bacteria. Chitinases and chitosanases belong to the PR proteins (pathogenesis related) [12]**.** Synthesis of these enzymes are induced when the plant are attacked by phytopathogens. These chitinases are induced by the growth regulators like ethylene which also acts as a pathogen bio control.

It also involves in the control of insect pests in transgenic plants. Molecular weight varies from 20 to 40 kDa[16]. Plant chitinases are tissue specific and are synthesized when they are induced by infection of phytopathogens[28]. Transgenic resistant plants were developed to induce the resistance against phytopathogens. They are also found in fungi *Trichoderma*, *Myrothecium,Penicillium*, *Beauveria*, *Lecanicillium*, *Neurospora*, *Lycoperdon*, *Aspergillus*, *Stachybotry*, *Conidiobolus*, *Metharhizium*, *Mucor* and *Agaricus.*More than ten isolates of *pseudomonas fluorescens* was isolated from barley and sugar beet rhizosphere and its antagonistic activity towards plant pathogens *Rhizoctonia solani* and *Pythium ultimum*, was described [33].

MAMMALIAN CHITINASES

These chitinases belongs to the glycosyl hydrolases family 18. These enzymes are sub divided into chitinases like protein and true chitinases. True chitinases are involved in the chitin hydrolyzing activity but in case of chitinases like protein, they just involved only in binding of chitin. The latter do not have enzymatic activity [16].Chitotriosidase is a first human chitinase identified in gaucher patients. This enzyme is produced by macrophages and it has good antifungal properties. Chitinases were found even in human such as chitotriosidase and acidic mammalian chitinase [39].Humans express chitin degrading enzymes, known as chitinases but they don't biosynthesize.Two known human chitinases that have chitinolytic activity, acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT-1), also many non- catalytically active chitinases called chi-lectins [45].

MICROBIAL CHITINASES

Chitinase production from microorganisms is higher when compared to higher organisms [28].

Fungal chitinases

Fungal chitinases belongs to family 18 and it consists of five domains namely N-terminal signal peptide region, chitin-binding domain, catalytic domain, C-terminal extension region, and serine/threonine-rich region. Last 3 domains are not necessary for chitin degrading activity and most chitinases lack these three domains. In fungi, chitinases play a major role in nutrition, morphogenesis and autolysis. Fungal chitinases are naturally inducible. NAG and Chitin acts as an inducers whereas glucose and other carbon sources acts as a repressor [8].Fungal strains *Trichoderma harzianum* and *Aspergillus niger* are also prospective chitinase-producing strains [39].

Bacterial Chitinases

Based on the amino acid sequence of individual catalytic domains, bacterial chitinases are separated into three major subfamilies A, B, and C. Subfamily A chitinases have the presence of a third domain corresponding to the insertion of an α+β fold region between the seventh and eighth (α/β)8 barrel [4]. Based on the molecular weight, chitinases are further classified into I, II, III, IV, V classes.Chitinases are widely distributed in bacteria such as *Streptomyces*, *Aeromonas*, *Chromobacterium*, *Klebsiella*, *Pseudomonas*, *Vibrio*, *Arthrobacter*, *Beneckea*, *Clostridium* and *Serratia*[39]. Family 19 are found recently in Actinobacteria species. Genus Streptomyces is a very good source of enzymes which are commercially produced and also a source of many novel bioactive compounds [27].Chitinolytic bacteria like *clostridium sp.* found in the feces of wild and domestic herbivores and also in the rumen fluids of cow. Molecular weight of chitinases varies from 20 to 80 kDa. Optimum pH and temperature varies from 5 to 8 and approximately 40º C respectively [2]**.**

CHITINASES PRODUCTION FROM *Streptomyces sp.*

Actinobacteria from marine source are widely distributed in biotic sources such as fishes, molluscus, sponges, seaweeds, mangroves, in addition to seawater and sediments [26].

Streptomyces sp. ANU 6277 was isolated from the laterite soil was 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 [31].*Streptomyces* sp. M-20 was isolated from the mangolian soil and the crude extract was purified and characterized. The molecular mass of chitinase was exhibited as 20 kDa by SDS PAGE [20]. *Streptomyces canus*, *Streptomyces pseudogriseolus* and *Micromonospora brevicatiana* were isolated from the marine sourceand optimized. *S. canus*and *M. brevicatiana* showed maximum activity from 40°C to 60°C and *S. pseudogriseolus* showed maximum activity from 40°C to 50°C. Optimum activity was at pH 8 [25]. Actinomycetes isolated from the cerrado soil in Brazil were tested for its endo-chitinolytic activity. Chitobiose and exo-chitinase was also detected. They were identified as *Streptomyces sp*. and it showed antagonistic activity against phytopathogenic fungi [13].*Streptomyces aureofaciens* CMUAc130, 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. Addition of starch, carboxy methyl cellulose and divalent cation like Mg^{2+} increases the chitinase production and activity[41].*Streptomyces tendae* was isolated from the saline soil in Riyadh city. The optimum parameter for chitinase production 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[29].

Actinobacteria strains were isolated from the shrimp and crab shell disposable area was detected for its chitinolytic activity colloidal chitin agar medium. There is a possibility in future to use the strain in shrimp and crab waste management and recycling process and also in medical applications[21].

Streptomyces sp. MT7 was isolated from the loktak lake soil and it secretes three essential fungal cell wall lytic enzymes chitinase, β-1, 3- glucanase, and protease, and siderophores. It shows a broad range of activity against wood rotting fungi [30].*Streptomyces lydicus* WYEClO8 a broad range of antifungal bio control agent. optimum temperature for chitinase was 25°C to 30°C and highest enzyme activity was observed when colloidal chitin of 1% was used and strong repression was observed when glucose, arabinose, xylose, cellulose, raffinose was used [24].

PARAMETERS

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[29].*Serratia marcescens* QMB 1466 has a pH range of 4 to 7 and optimum temperature as 30º C [49]. Likewise, *vibrio alginolyticus* has a wide optimum pH range of 4 to 9 and optimum temperature for chitinase production is 40º C[50].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. Exo-chitinase which is thermostable was recovered from *Bacillus stearothermophilus* CH -4 from organic solid waste compost [15].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 [39].

SUBSTRATES USED

During the process of chitinase production, chitin acts as an inducer and also as carbon and nitrogen source. To achieve the required quantity of chitinase, chitin must be added to the medium to induce the production or else it will be produced in very low quantity during growth.Like chitin, some of the other carbon sources for inducing chitinases production are chitobiose, chitooligosaccharides, glucosamine. Colloidalchitin and cell wall of fungi are also utilized as carbon source. It was reported that chitinase production was increased while using colloidal chitin [28].Presence of chitoligomers is necessary in the medium which can directly supply or through hydrolysis of colloidal chitin. Chitin powder and chitin flakes poorly induce the chitinase activity. Compared to colloidal chitin, lactic acid processed chitin induces chitinase better [39].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. Whenglucose was used along with chitin, catabolite repression was observed [4].Actinomycetes and some microorganisms use shrimp shells as substrate for chitinase production where it utilizes shells more efficiently than colloidal chitin. *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.

MEDIUM OPTIMIZATION

Statistical methodologies were applied to optimize the medium components for the production of chitinase by *Chitinolyticbacter meiyuanensis* SYBC-H1 isolated from soil. Compared to the unoptimized medium there was 15.5 folds increase in the production of chitinase in optimized media [18].*Lysinibacillus fusiformis B-CM18* was isolated from chick pea rhizosphere was investigated for its chitinase production and also required parameters were optimized by response surface methodology. There was an increase in the chitinase production of 56.1 fold with optimized conditions [38].

FERMENTATION

Chitinases are produced by batch, fed batch and continuous fermentation [4].

MONOCULTURE

It is difficult to compare the chitinase production in literatures because there is not any standard combined method to detect the chitinase activity since there are different ways to hydrolyse the source of chitinase [39].Some of the bio reactors are used to produce chitinases in liquid medium. They are stirred tank reactors, air lift reactors, bubble column reactors, air lift with net draft tube. High agitation rates create a shear stress condition in stirred tank reactors. This affects the chitinase production and this can be overcome by using airlift bioreactors. Very good option to increase the chitinase production is switching to continuous cultivation mode. Production and activity of chitinase was increased when stirred tank reactor was used with continuous mode for cultivation. Live immobilized cells are used to reduce the cost in stirred tank reactors.The novel self-directing non-statistical optimization of parameters for the production of extracellular chitinase was done in batch mode by using *Trichoderma harzianum.* The enzyme activity of an extracellular chitinase was increased to 0.384 U after optimization of suitable grouping of parameters [10]. Production of chitinase by continuous mode of fermentation by using *Paenibacillus sp.* CHE-N1 in a membrane bioreactor was studied. Crab shell chitin powder was used as a media source. Enzyme activity of chitinase in membrane reactors was 78 % higher than batch mode of operation [19]. The investigation for production of chitinase by *Verticillium lecanii* in submerged fermentation using both shaker flasks and large scale bioreactors were studied. Enzyme activity in shaker flasks was 9.95 mU/ml. The chitinase activity was 18.2 mU/ml with 5 litre stirred tank bioreactors and with 30 litre airlift bioreactor the activity of enzyme was increased to 19.9 mU/ml [23]. *Beauveria bassiana*, a chitin degrading fungi which was isolated from the sediments of sea water was optimised for the optimum process parameters using wheat bran as a substrate in solid state fermentations. The maximum yield of chitinase from marine fungus is 246.6 U/g of dry substrate [40]. Production of chitinase from isolates of soil which is rich in chitin from drying fields of shrimp was studied. The production of chitinase from two isolates of *Penicillium chrysogenum* was optimized and yield of two isolates were 3809 U/g dry substrate and 2516 U/g dry substrates respectively [35]. Solid substrate fermentation has recently gained importance for the production of microbial enzymes due to several economic advantages over conventional submerged fermentation. Wheat bran with crude chitin flakes was used as a substrate for the production of extracellular chitinase by *Penicillium aculeatum* NRRL 2129 in solid state fermentation [1].

CO-CULTURE

Microorganisms are found to be more efficient when they are allowed to grow together with other microorganisms of same or different species [43].The best part in co-culture is precise quality control and better yield of the product can be achieved with low cost management by utilizing cheaper substrates. Many enzymes like cellulases, ligninases, amylases, laccases, lipases, xylanases are produced in higher amounts by using co-culture. In case of chitinase enzyme, to the best of my knowledge there was not any previous reports on the production of chitinases using co-culture.

PRODUCTION OF CHITINASE ENZYME BY RECOMBINANT MICROORGANISMS

In fermentation process, there was an enhanced production of desired products by using recombinant microorganisms [9]. Recombinant *E.coli* holding a chitinase gene from *Aeromonas hydrophilia* for enhanced production of chitinase [3]. IPTG is used to induce the chitinase expression. Production of chitinase enzyme was non-growth associated.

NOVEL IMMOBILIZATION IN THE PRODUCTION OF CHITINASE

It is a novel method for the production of chitinases. *Micromonospora chalcae* was used both in free and immobilized form [9]. It was immobilized using calcium alginate with chitin. Chitinase production in immobilized cells was 0.9 U and its activity lasts for long time but in free cells maximum activity was 0.6 U and its activity decreased after three days [34]. Chitinase production from *Wasabia japonica* cells was immobilized on double layered gel fibres. The concentration of chitin must be maintained below 2 U to avoid product inhibition so that 80% maximum chitinase production can be attained [42]. *Pseudomonas aeruginosa* K-187 isolated from soil of Taiwan was grown in medium containing shrimp and crab shells powder. This strain was immobilized on a polymeric

support which has 99% efficiency of immobilization. Immobilized chitinase holds on its 70% activity after 10 batches of chitin degrading activity [47].

APPLICATIONS

Major application includes photographic products, cements, chelating agents of heavy metals, cosmetics [15].In most cases, chitinases from bacterial source acts as a fungicidal compound. Actinomycetes also display strong fungicidal properties. *S. griseus, Streptomyces halstedii,* and *S. cavourensis* produce highly active antifungal CHIs, which suggests the opportunity of using them as agents for biological protection of crops. 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[2]. The biodegradable and antifungal properties of chitinase are also useful for environmental and agricultural uses, food technology and cosmetics [26].

Production of single cell protein

Chitin present in the solid waste from shell fish was converted to single cell protein by chitinolytic enzymes. Some of the fungal source for the production of SCP is *Saccharomyces cerevisiae,Candida tropicalis, Hansenula polymorpha,* and *Myrothecium verrucaria*. Best reports for the production of chitinases are from *S.cerevisiae* where more than 60 % SCP was produced and with less nucleic acid content (1% to 3 %) [4].

Bio control of phytopathogens

Use of microorganisms or their enzymes to control the insect pests or phytopathogens offers a significant approach for agriculture. By using chitinases along with insecticides and fungicides, decreases the concentration of chemical content of these pesticides and fungicides [4]. Chitinolytic bacteria also showed suppressing abilities for plant pathogens, such as *Paenibacillus* sp. and *Streptomyces sp*. against Fusarium wilt of cucumber (*Cucumis sativus*) caused by *Fusarium oxysporum f. sp. cucumerinum* [39] .The destruction of causative agent of witches broom disease of cocoa, *Crinipellis perniciosa* by using partially purified chitinase from *T.harzianum* was investigated [7].Chitinase from yam was used as a bio control agent for powdery mildew in strawberry.in this paper they have developed transgenic plants resistant to fungal pathogens. It is also used as a bio control agent against insect pests which affects pine tree and causes pine sawyer beetle [5].

Isolation of protoplasts

To study cell wall synthesis, enzyme secretion, enzyme synthesis and strain improvement fungal protoplast are used very effectively. Since, most fungi has chitin layer in the cell wall, so chitinolytic enzymes like chitinases are vital for degradation of cell wall for formation of protoplast. Protoplast was isolated from *Schizophylum commune* using the enzyme complex in culture filtrate of *B.circulans* [48].

Chitinase from Streptomyces was found to lyse effectively the hyphal walls of *A.oryzae* and *Fusarium solani*[9].

Fungal biomass estimation

There is a great relation between fungal growth in soil and activity of enzyme chitinase. Chitinase is an indicator for fungi growth in soil. Similarly, fungal infection in human can also be detected by chitinase and chitinolytic enzymes [4].

Bio pesticides

Inhibitors of chitinases are found to be potent bio pesticides. Chitin is present in gut lining of insects. Allosamidin which inhibits the growth of housefly larva and mite is a potent inhibitor of chitinases [4].

Production of oligomers

GlcNAc, chitooligosaccharides, glucosamine have a very essential role in pharmaceuticals. Chitooligosaccharides is used even in human medicines. Chitinolytic enzymes are used in different combinations to get the desired oligomers [4].

Mosquito control

Aedes aegypti is a vector of dengue and yellow fever. This can't be infected by even entomopathogenic fungus. But when purified chitinase was used for lipid degrading activity, time required decreased to 24 hours from 48 hours and 48 hours from 120 hours for first and fourth instar larva of mosquito [4].

Medical applications

Chitinases are used along with antifungal drugs to increase the efficiency in treating the fungal infections. Chitinases are also used as additives in skin lotions and antifungal creams[4].The antifungal activity and highly biocompatible quality make chitinase and its derivatives particularly useful for biomedical applications, such as wound healings,

drug delivery, cartilage tissue engineering and nerve generation [26]. Chitin and chitosan are used as membrane for drug delivery and also in tissue engineering. This in turn increases the need of highly purified enzymes. Chitinases are also used in anti-cancer therapy.

Allergic inflammation

Though mammals cannot synthesize or metabolize chitin, a number of true chitinolytic enzymes like chitotriosidases, acidic mammalian chitinase (AMCase) or chitin-binding proteins or chitinase-like proteins (CLPs) such as Ym-1, Ym-2, and breast regression protein 39 were discovered in mammals. Thus, whether the chitotriosidase in humans has the same immunologic activity in allergic responses as AMCase in mouse is an interesting query that needs to be investigated in further studies [22].

Chitinase in degradation of fish waste

NAG, monomer of chitin is used in the manufacture of food products such as sweeteners, growth factors, chemicals, pharmaceutical intermediates[9].The chitinase activity in bioconversion of shell fish waste to NAG [44]

CONCLUSION

Recombinant techniques can be used to increase the production of chitinases for industrial purposes. Protein engineering can be used to produce chitinases with desired functions to meet the requirements of chitinases for treating various disorders. Reactors which supports the enhanced production of chitinases with appropriate parameters should be choosed. Since,carbon sources has a major influence in the enzyme productions, suitable carbon sources must be utilized. Influence of aeration, agitation rate on chitinase production is not given much importance. So scale up is difficult without full information about the required parameters. Not much research was done using airlift and bubble column reactors.

REFERENCES

[1] P Binod; T Pusztahelyi; V Nagy; C Sandhya; G Szakacs; I Pocsi; A pandey, *Enzyme Microb. Technol.*,**2005**, 36(7), 880-887.
[2] MS Brzezinska; U Jankiewicz; A Burkowska; M Walczak,*Curr. Microbiol*.,**2014**,68(1),71-81.
[3] SJ Chen; MC Chang, CY Cheng,*J. Ferment. Bioeng*.,**1997,** 84(6), 610-613.
[4] N Dahiya; R Tewari; GS Hoondal,*Appl. Microbiol. Biotechnol.*,**2006**, 71(6), 773-782.
[5] K J Daizo,*Metals Mater. Min*, **2005,** 15(1), 33-36.
[6] MP Das; L J Rebecca; S Sharmila; Anu; A Banerjee; D Kumar,*J. Chem. Pharm. Res*.**, 2012**, 4(11),4816-4821.
[7] JL De Marco; L H C Lima; MV de Souza; C R Felix, *World J. Microbiol. Biotechnol.*,**2000,** 16(4), 383-386.
[8] L Duo-Chuan,*Mycopathologia*,**2006,** 161(6), 345-360.
[9] P Felse; T Panda,*Bioprocess Eng.*, **2000,** 23(2), 127-134.
[10] P A Felse; T Panda,*Process Biochem.,* **1999**, 34(6), 563-566.
[11] LF Fleuri; HY Kawaguti;HH Sato,*Braz. J. Microbiol.*,**2009**, 40(3), 623-630.
[12] T Fukamizo,*Curr. Protein Pept. Sci.,* **2000,** 1(1), 105-124.
[13] RGomes; L Semedo; RMA Soares; CS Alviano; LF Linhares; RRR Corlho,*Lett. Appl. Microbiol.*,**2000**,30(2), 146-150.
[14] SH Guo; J K. Chen; WC Lee,*Enzyme Microb. Technol.*,**2004**, 35(6), 550-556.
[15] G Haki; S Rakshit,*Bioresour. Technol.*,**2003,**89(1), 17-34.
[16] R Hamid; M A Khan; M Ahmad; M M Ahmad et al, *J. Pharm. Bioall. Sci.,* **2013**,5(1), 21.
[17] YHan; B Yang; F Zhang; X Miao; Z Li,*Mar. Biotechnol.*,**2009**, 11(1), 132-140.
[18] Z Hao; Y Cai ; X Liao; X Zhang; X Fang et al, *Braz. J. Microbiol,* **2012**, 43(1), 177-186.
[19] PM Kao; S C Huang; YC Chang; YC Liu,*Process Biochem.*, **2007**, 42(4), 606-611.
[20] KJ Kim, Y J Yang; JG Kim,*J. Biochem. Mol. Biol.*,**2003**, 36(2),185-189.
[21] S Kumaran; B Deivasigamani; U Vairagkar; S Balamurugan; M sakthivel,*Asian Pac. J. Trop. Dis.*,**2012**, 2, S861-S864.
[22] CG Lee,*Yonsei Med. J*.,**2009,** 50(1), 22-30.
[23] BL Liu; PM Kao; YM Tzeng; KC Feng, *Enzyme Microb. Technol*., **2003**, 33(4),410-415.
[24] B Mahadevan; DL Crawford, *Enzyme Microb. Technol*., **1997,** 20(7), 489-493.
[25] U Mane; A Deshmukh,*Afr. J. Biotechnol.*,**2009,** 8(23).
[26] P Manivasagan; J Venkatesan; K Sivakumar; SK Kim,*Microbiol. Res*,**2014**, 169(4), 262-278.
[27] A Marmann; AH Aly; W Lin; B Wang; P Proksch,*Mar. Drugs*, **2014,** 12(2), 1043-1065.
[28] KS Matsumoto,*Adv. Agric. Food Biotechnol*.,289.
[29] S Meena; R K Gothwal;J Saxena; M K Mohan; P Ghosh,*Ann. Microbial*,, **2014**, 64(2), 787-797.
[30] A Nagpure; B Choudhary; S Kumar; RK Gupta,*Ann. Microbial*,**2014**,64(2), 531-541.

[31] KJ Narayana; M Vijayalakshmi,*Braz. J. Microbiol*,**2009**,40(4), 725-733.
[32] N Nawani; B Kapadnis; AD Das; AS Rao; SK Mahajan, *J Appl. Microbial.*,**2002**, 93(6), 965-975.
[33] MN Nielsen; J Sørensen, *FEMS Microbiol. Ecol.*,**1999**,30(3),217-227.
[34] A O'Riordan; M. McHale; AO Riordon; J Gallagher; AP McHale,*Biotechnol. Lett*,**1989**, 11(10), 735-738.
[35] P Patidar; D Agrawal; T Banerjee; S Patil,*Process Biochem.*,**2005,** 40(9), 2962-2967.
[36] RS Patil; V Ghormade;MV Deshpande,*Enzyme Microb. Technol*,**2000**, 26(7), 473-483.
[37] S Ramyasree; JR Dutta,*Biocatal. Agric. Biotechnol*,***2013***, 2(4), 393-398.
[38] RK Singh; DP Kumar; MK Solanki; P singh; AK Srivastva; S Kumar; PL Kashyap; AK Saxena; PK Singhal; DK Arora,*J. Basic Microbiol.*, **2013,** 53(5), 451-460.
[39] YM Stoykov; AI Pavlov;AI Krastanov,*Eng. Life Sci.*,**2015,**15(1), 30-38.
[40] P Suresh; M Chandrasekaran,*Process Biochem.*,**1999**, 34(3), 257-267.
[41] T Taechowisan; J Peberdy; S Lumyong, *Ann. Microbial.*, **2003,** 53(4),447-462.
[42] H Tanaka; Y Kaneko; H Aoyagi; Y Yamamoto; Y Fukunaga,*J. Ferment. Bioeng.*,**1996,** 81(3), 220-225.
[43] A Tesfaw; F Assefa, *Biotech. & Mol. Biol. Review*,**2014**,9 (2), 12 20.
[44] RA Tom; PA Carroad,*J. Food Sci.*, **1981** 46(2), 646-647.
[45] K Vega; M Kalkum,*Int. J microbiol.*,**2011.**
[46] S R Waghmare; J S Ghosh,*Carbohydr Res*, **2010**, 345(18), 2630-2635.
[47] S L Wang; S H Chio, *Enzyme Microb. Technol*, **1998**, 22(7), 634-640.
[48] S Yano; M. Wakayama; T Tachiki,*Biosci. Biotechnol. Biochem.*,**2006**, 70(7), 1754-1763.
[49] RL Roberts; E Cabin, *Anal. Biochem.*, **1982,** 127, 402-412.
[50] S Murao; T Kuwada; H Itoh; H Oyama; T Shin,*Biosci. Biotechnol. Biochem.*, **1992,**56, 368-369.