OPTIMIZATION OF CHITINASE EXTRACTON BY *Streptomyces hygroscopicus* DSCH 2 ISOLATED FROM THE SAMPLE OF SOIL

PRIYA C. S.[1]*, DEIVASIGAMANIB.[1]

[1]Centre of Advanced Study in Marine Biology,
Faculty of Marine Sciences, Annamalai University,
Parangipettai 608502, India.

ANUSHA N [2#] (SHAJAHAN A) [2]

[2]Department of Biotechnology,
PRIST University, Pondicherry (campus). 605007.

#Co-auther :E-mail: nedunchezhiyananusha@gmail.com

ABSTRACT

A total of 10 chitinolytic *streptomycetes* were isolated from 40 soil samples collected from completely different elements of Tamil Nadu, India. Among them, a strain selected as DSCH2 [Deivasigamani chennai strain a 2] that made highest polysaccharideolytic activity. In primary and secondary screening in mixture chitin agar was elect and later known as *Actinomycete hygroscopicus*. In our previous study, the assembly of chitnase was administrated by *Actinomycete hygroscopicus* VMCH2. The assembly of chitinase by *Actinomycete hygroscopicus* VMCH2 was optimised victimization completely different growth media, substrate concentration, pH, temperature and period. The utmost polysaccharidease production was ascertained in CCMB (colloidal chitin medium broth) amended 0.1 % mixture polysaccharide at hydrogen ion concentration 7.0 and 35° C when eight days of immunization. Below these optimized growth conditions, *Actinomycete hygroscopicus* VMCH2 made a complete chitinase activity of 50.05 units /ml as against solely 13.4 units/ml within the initial CCMB production medium, that could be a 4-fold increment. This study is to decrease the period from 8 days to 6 days because the strain is from coastal region.

Keywords: *Streptomyces hygroscopicus*, chitinolytic bacteria, chitinase, colloidal chitin, inhibition.

INTRODUCTION

Chitin, a linear β 1, 4 coupled homopolymer of N acetylglucosamine is one in every of the three most overabundant polysaccharides within the nature, besides polysaccharide and starch. The presence of polyose within the cyst wall of human microorganism, Entamoeba was explained (Das *et al*., 2006, Chakraborthy *et al*., 2012). The polyoseases that are referred to as hydrolytic enzymes that specifically degrade chitin,

became a lot of necessary (Haggag *et al*., 2012). As for bacterium, chitinases play a crucial role in nourishment, interdependency and polyose recycle. In fungi, protozoa and invertebrates and humans, chitinases recognized defensive mechanisms against pathogens (Shanmugaiah *et al*., 2008). Chitinolytic enzymes are widely used, for the preparation of crucial chito-oligosaccharides and N-acteyl D- glucosamines are the single cell macromolecule or within the method of isolation from fungi. Moreover, they are used in restricted area needed for his or her cultivation, longer period of time; the benefit with them is often genetically manipulated to get improved enzymes. Wide selection of microorganisms like viruses, fungi, bacterium is proverbial to provide chitinases. *Actinomycetes* are documented producers of chitinases (Kumar and Singh 2013, Mohanta, 2014). Individuals did researches to explore unknown habitats to get new *Actinomycetes* strains to provide novel chitinase catalyst, having applications in numerous industries (Gurung *et al*., 2013, Anbu *et al*., 2015). Once this exploration of the fermentation processes to boost the assembly and purification of quality enzymes from the proper strain on an oversized scale (Bui 2014, Yassien *et al*., 2014, Kumar *et al*., 2016) are going to be done. Because of increase in industrialization and urbanisation, selling of monumental quantity of waste materials has become a nuisance (Akhtar 2014). Typical techniques for management of perishable wastes are getting more and more overpriced and energy inefficient. The chemical treatment ways are dangerous to each setting and humans (Wilts *et al*., 2016). Since, chitinases are in demand for his or her application in numerous fields like antifungal biocontrol activity, biotechnology, food, medicine, waste product treatment and agriculture trade. There are some limitations for the applying of chitinases on the business scale like low chitinase yields, high production time and low chemical action activity of the offered catalyst thus low polyose degradation. The aim of this study was to isolate animate thing chitinase manufacturing *Actinomycetes* and optimizing fermentation conditions for the high production of the chitinase.

MATERIALS AND METHODS

1. Sample assortment and isolation of *Actinomycetes*

A complete of 40 totally completely different soil samples were aseptically collected from different regions of Chennai. The soil was collected from coasts of Chennai, fish market, *Rhizosphere* of maize, wheat and rice. *Actinomycetes* population from the soil were determined by serial dilution and unfold plating technique on mixture polysaccharide agar (CCA). 10 grams of soil sample was taken associated transferred to an Erlenmayer flask containing 90ml of sterile water. The soil sample was serially diluted upto10 levels. Aliquot (1ml) from serial power dilution of every suspension was pipetted into the CCMB containing 1% chitin 1% of the inoculum was inoculated to the medium and incubated at150rpm during a rotary shaker at 35°C for five days. Once the time period, the cultures were harvested, centrifuged at10000rpm for ten minutes at 4°C. The cell free supernatant of 100µl was placed into the wells on CCA plates and incubated at 37°C. Once twelve hours, the event of the clear zone was ascertained.

2. Identification of designated *Actinomycetes* strain

Among 10 designated *Actinomycetes* strains, there was a particular strain with high activity of chitinase. it had been known on the idea of organic chemistry tests still as on the analysis of the sequence of genes coding 16S rRNA sequencing was distributed exploitation the universal forward primer: GGA TGA GCC CGC GGC CTA. Reverse primer: CGG CCG CGG CTG CTG CTG ground-controlled approach CGT. Under the subsequent conditions;

i. Initial denaturation 94°C for 5second (35 cycles)
ii. Denaturation 94°C for 60 seconds;
iii. Annealing 55°C for 60 seconds;
iv. Extension 72°C for 90 seconds;
v. Final extension 72°C for 5 seconds;

Around 240bp of PCR product was directly sequenced employing a sequencing kit on an automatic sequencer. Similarity of the 16S rRNA sequence of the isolate was analysed by exploitation BLAST programme from information bank knowledge base. The sequencing was tired action labs, Chennai.

3. Optimization of culture conditions

Cultivation of *Actimomycetes* was performed on the media containing totally different sources of Carbon and Nitrogen gas. Medium consisted of (gm / l);

i. Chitin – 10,
ii. glucose-7.5,
iii. $NaNO_3$-2.0,
iv. K_2HPO_4-1.0,
v. $MgSO_4.7H_2O$ -1.0,
vi. $CaCO_3$- 1.0,
vii. $FeSO_4.7$, H_2O-0.01,
viii. KCl- 0.5,
ix. H_2O-1000ml,
x. pH7.

Chitinase activity was measured in terms of the quantity of N-acetyl-D-glucosamine sugars free from polysaccharide by the chitinase action as delineated mistreatment p- DMAB as a colouring chemical agent. Accelerator units were expressed in international units (IU) as micromoles of N- acetyl glucosamine free by 1ml of accelerator in 1 minute beneath the assay conditions. Optimization of the chitinase production was done by varied totally different physico-chemical factors. Result of incubation time on accelerator production and growth. The result of incubation time on accelerator production was studied by retreated the

samples from the culture at totally different time intervals upto 192 hours and doing the assay mistreatment cell free supernatant. The result of pH scale on accelerator production was studied with totally different pH scale starting from 5.0 to 7.5 and doing the assay mistreatment cell free supernatant. The result of incubation temperature on accelerator production was studied at totally different temperature starting from 18°C to 40°C. The result of substance size was studied at totally different substance concentration starting from 0.1 - 1.2%. The result of various carbon supply viz. Starch, glucose, fructose, chitin, brain sugar and plant product was studied at 0.5% concentration. The result of various gas sources viz. mixture polysaccharide, peptone, yeast extract, NH_4SO_4, beef extract, NH_4NO_3, gelatin and carbamide was studied at 0.1%. The result of polysaccharide was studied at totally different concentration starting from 0.1-1.5%. The result of mixture polysaccharide was studied mistreatment totally different concentration starting from 0.1-1%

RESULTS AND DISCUSSION

A complete of 10 isolates were obtained from soil samples that were enriched in CCMB fortified with 1% chitin powder. Primary screening for isolates on mixture polysaccharide agar plates incontestible the chitinolytic ability of the isolates. Among five isolates, one isolate that created highest zone of clearance was hand-picked for additional studies. The morphological and organic chemistry characterization was performed to spot the chosen isolate. Morphological and organic chemistry characteristics of the isolate hand-picked for additional studies are summarized in table 1-4. (Influence of culture conditions on chitinase production and investigation of the result of further carbon source). The expansion and production of chitinase by the isolate was investigated victimisation totally different carbon sources. Amongst all the carbon sources used, mixture polysaccharide served because the best carbon supply for the assembly of chitinase. Carbon sources like aldohexose, sucrose, water pill and Glc North Atlantic Council served to inhibit chitinase synthesis in spite of supporting prosperous cell growth of the isolate within the medium. Fig. two shows the most production of chitinase at 0.1% concentration.

Investigation of the result of further element supply, the study was performed victimisation 2 organic (peptone and tryptone) and 2 inorganic (ammonium salt and ammonium ion chloride) element sources, that were more within the medium that contained mixture polysaccharide as a sole supply of carbon. Amongst the element sources investigated, organic compound served as best element supply for production of chitinase. Optimisation of the initial hydrogen ion concentration of the medium (Fig. 4) shows the influence of initial hydrogen ion concentration on the matter. Most chitinase production was discovered over a variety of hydrogen ion concentration 6-7. At hydrogen ion concentration higher and less than the optimum hydrogen ion concentration vary, protein production was pent-up. Optimum temperature for protein production (Fig. 1) shows the influence of temperature on chitinase production. Most chitinase production was discovered at the various temperature of 30-35°C. Increase within the temperature resulted in decrease in protein production. These results show the organism's preferance for protein production. Optimisation of $MgSO_4$ concentration was discovered that with the rise within the concentration of $MgSO_4$, the strain synthesised a lot of chitinase. The most level of chitinase remained constant in spite of increase in $MgSO_4$

concentration. The optimisation of substrate concentration is given within the fig-2. Chitinase production was discovered with relation to substrate concentration, the optimum level discovered was within 0.50- 1%.

In the current study, soil samples were screened for chitinase manufacturing organisms on selective medium containing mixture chitin. The isolated organism from the higher than mentioned soil samples was hand-picked for additional studies thanks to its formation of largest zone of clearance on mixture polysaccharide agar plate. The morphological and organic chemistry characteristics of the isolate were investigated and it indicated that the isolate belonged to bacteria genus. The isolate beneath investigation was attributed to bacteria genus on the premise of 16S rRNA sequencing and therefore the species hygroscopicus by reference technique. It's imperative to optimize the matter so as to confirm optimum growth of microorganisms and production of metabolites. The study of result of further carbon supply 1%(w/v) on chitinase production incontestible that no chitinase activity was discovered within the medium that was supplemented with further carbon sources like aldohexose, glucose, sucrose, mannitol and Glc NAc in spite of prosperous cell growth of the isolate within the medium. This finding indicated that polysaccharide or mixture polysaccharide was indispensable for polysaccharidease production that coincides with previous reports that recommend using mixture chitin as sole carbon supply for highest chitinase production (Dhar P and Kaur G 2010). The study conjointly supports the idea that chitinase is subjected to stimulatory result and catabolite repression by sugars. Previous report by (Twedell *et al.*, 2003) suggests no chitinase production once aldohexose, saccharose or Glc NAc was used as carbon sources. Similar findings report decrease in chitinase production by M.timonae and bacteria species once ketohexose, glucose, lactose, disaccharide and GlcNAc was used as carbon sources within the medium (Farmarzi *et al*., 2009, Chakraborthy *et al*., 2012).

Chitinase production is additionally tormented by the presence of minerals within the production medium. The concentrations of Mg^{2+} ions play an important role in cell growth and protein production and stability. The study of optimisation of $MgSO_4$ concentration incontestible a positive result on chitinase production. This is often agreement with (Gohel *et al*., 2005 and Han *et al*., 2008) that reports positive influence of $MgSO_4$ on chitinase production by *B. pumilus*. (Tasharro *et al*., 2011). The influence of environmental factors like hydrogen ion concentration and temperature has an impression over biological processes like protein production by dominant the supply of silver ions. Majority of microorganism are reported to provide most chitinase at neutral or slightly acidic hydrogen ion concentration (Mathivanan *et al*., 1998). In *Bacillus globigii* hydrogen ion concentration seven and eight are reported to be optimum for chitinase production (Karunya *et al*., 2011). Similarly, neutral hydrogen ion concentration is reported as optimum hydrogen ion concentration for chitinase production by different strains of *Bacilli* and genus *Pseudomonas* (Purwani *et al*., 1997). Optimum temperature is crucial in production of chitinase since it influences cell growth. The observations as a result of the optimum temperature study is in complete agreement with (Das *et al*., 2012) that reports 35°C because the optimum temperature for chitinase production by *B.amyloliquefaciens* SM3 strain (Karunya KS). Previous report on *B.laterosporus* conjointly states 35°C as the optimum temperature for chitinase production. The optimum concentration of chitin, the substrate within the production medium is crucial for chitinase induction so as to provide most chitinase. Six

totally different polysaccharide concentrations (0.05%-3%w/v) were investigated for polysaccharidease production and therefore the observations coincides with the report by (Taechowisan *et al*., 2003) that states that polysaccharidease created by microorganism hydrolyzed mixture polysaccharide sooner than crude chitin or chitin from fungal cell walls.

CONCLUSION

The present study suggests the potential use of the obtained isolate in production of polysaccharidease victimisation polysaccharide or chitin derivatives as stuff. a close study is necessitated so as to harness the power of the isolate to provide industrial price more product. Within the course of the study it absolutely was established that matter and method parameters play an essential role in production of enzymes. The pilot scale study is adopted so as to maximise the assembly of chitinase for additional application.

FIGURES AND TABLES

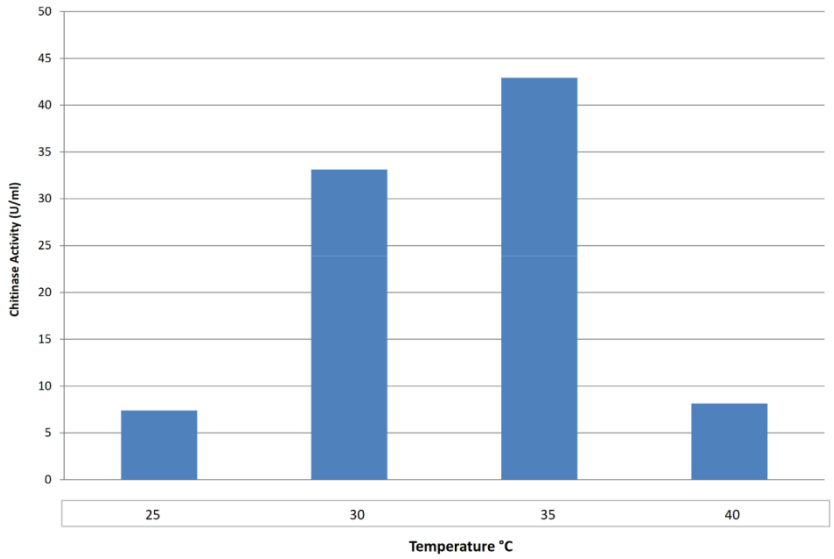

Fig: 1.Shows the chitinase activity with different temperatures

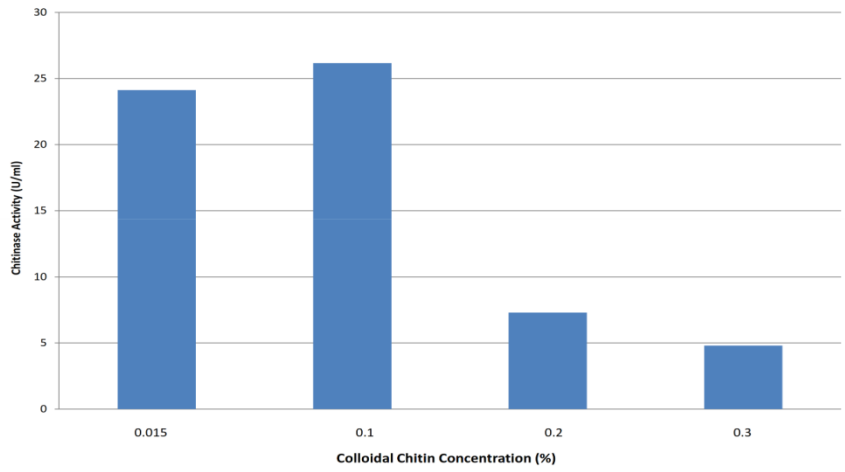

Fig: 2. Colloidal chitin concentration varying activity of chitinase

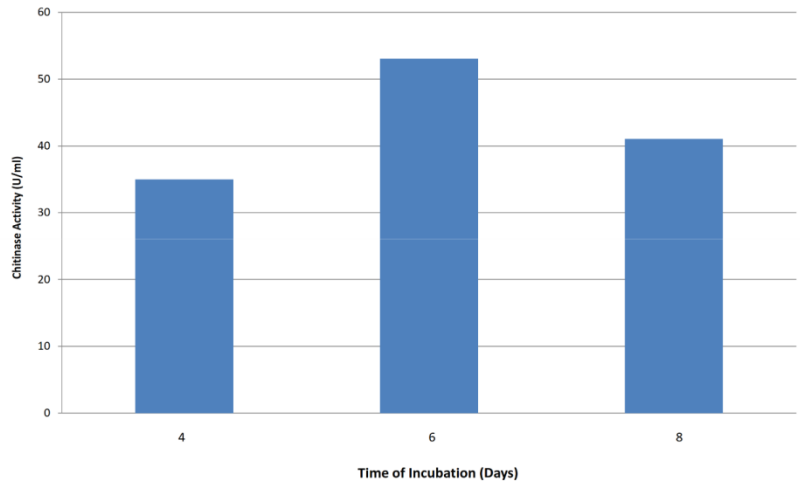

Fig: 3. Changes in the Chitin during experimental days

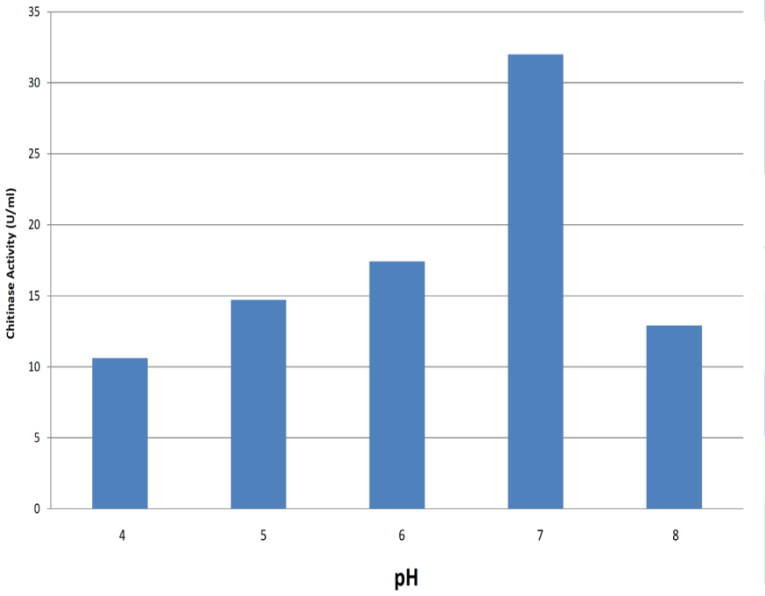

Fig: 4. Changes of chitinase activity depend on the pH values

TABLES

Biochemical test	Temperature	Characteristics
Gram staining	11° C	+
Spore staining	20° C	+
Starch hydrolysis	15° C	+
Catalase hydrolysis	25° C	+
Oxidase activity	37° C	+
Methyl Red (M-R) test	42° C	+
Indole production	36° C	+
Citrate utilization	39° C	+

Table: 1. Percentage growth inhibition of the rice phytopathogens by the indigenous bioactive *actinomycete* isolates % of mycelial growth inhibition

Isolates	Phospate solubilisation	Chitinase production
DSCH - 2	+	+
FMCH - 1 -9	+	+
FMCH - 10 - 20	++	+
FMCH - 21 – 40	+	+

Table: 2.

Biochemical, morphological and physiological characteristics of DSCH – 2

Biochemical test	pH	Characteristics
Urease production	7.0	+
Nitrate reduction	8.0	+
Gelatin liquefaction	7.5	+
H2S production	10.0	+
Growth in 7% Nacl	9.0	+

Table: 3. Effect of pH on growth

Carbon Sources	Characteristics	Nitrogen Sources	Characteristics
D – Glucose	+++	Phenylalanine	+
Fructose	+++	Gelatin	+
D - Mannose	+++	Valine	+
Beta methylxyloside	+	Hydroxyproline	+
Mannitol	+++	Casein	+
Sorbitol	++	Yeast extract	+
Maltose	+++	Malt extract	+
Rhamnose	+	Threonine	-
Saccarose	+++	Peptone	+
Sucrose	-	KNO3	+

Table : 4. Utilization of nitrogen sources, Utilization of carbon sources

The most potent biocontrol strain, DSCH – 2 was identified by phenotypic (Table. 3) and genotypic tests (16s rRNA sequence) analysis as S.*hygroscopious*. Next to DSCH – 2, FMCH 1, FMCH 3 – 6, FMCH 7 -10 other promising isolates were antagonistic against rice phytopathogens.

REFERENCE

Akhir SM, Abd – Aziz S, Salleh MM, Rahman RA, Medium Optimization of Chitinase Enzyme Production from Shrimp Waste Using Bacillus Licheniformis TH-1 by Response Surface Methods, Biotechnology 2009; 8:120 – 125.

Akhtar MN (2014). Prospective assessment for long-term impact of excessive solid waste generation on the environment. Int.J. Adv.Earth Environ.Sci.2(2): 39-45.

Anbu P, Gopinath SCB, Chaulagain BP, Tang TH, Citartan M (2015). Microbial enzymes and their applications in industries and medicine. Biomed.Res.Int.pp. 1-2.

Bui HB (2014). Isolation of cellulolytic bacteria including actinomycetes from coffee exocarps in coffee-producing areas in Vietnam.Int.J.Recycl. Org.Waste Agric. 3:48.

Chakrabortty S, Bhattacharya S, Das A. Optimization of Process Parameters for Chitinase Production by a Marine Isolate of Serratia marcescens.Int.J.Pharm.Biol.Sci.2012;2:08-20.

Das MP, Rebecca JL, Sharmila S, Anu A, Banerjee and D Kumar.Identification and optimization of cultural conditions for chitinase production by Bacillus amyloliquefaciens SM3. Journal of Chemical and Pharmaceutical Research 2012; 4:4816 -4821.

Dhar P,Kaur G..Effects of carbon and nitrogen sources on the induction and repression of chitinase enzyme from Beauveria bassiana isolates. Afr.J.Biotechnol.2010; 9:8092-8099.

Faramarzi MA, Fazeli M, Tabatabaei Y, Adrangi S, KJami Al Ahmadi, N Tasharro i and F Aziz Mohseni .Optimization of Cultural conditions for production of Chitinase by a Soil Isolate of Massiliatimonae. Biotechnology 2009;8:93 – 99.

Ghosh SK, Van Dellen KL, Chatterjee A, Dey T, Haque R, Robbins PW, Samuelson 2010. PLOS Negl Trop Dis.2010.

Gohel V , Jiwan D , Vyas P, Chhatpar HS. (Statistical optimization of chitnase production by Pantoea dispersal to enhance degradation of crustacean chitin waste)J Microbiol Biotechnol, 2005 ;15: 197 - 201.

Gohel V, Chaudhary T,Vyas P, Chhatpar HS. Statistical screening of medium components for the production of chitinase by the marine isolate Pantoea dispersa . Biochem.Eng.J.2006;28:50 – 56.

Gohel V, Singh A,MaisuriaV, Phadnis A, Chhatpar HS. Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr.J.Biotechnol.2006; 5:54 -72.

Gurung N, Ray S, Bose S, Rai V (2013). A broader view: Microbial enzymes and their relevance in industries, medicine and beyond. Biomed. Res.Int.1-18.

Haggag W and Abdallh E, Purification and Characterization of Chitinase produced by Streptomyces hygroscopicus against some phytopathogens. J.Microbiol.Res. 2012; 2:145-151.

Han Y, Zhiyong L, Miao X, Zhang F, Statistical optimization of medium components to improve the chitinase activity of Streptomyces sp. Da11 associated with the South China Sea sponge Craniella australiensis. Process Biochem.2008:43:1088 – 1093.

Ilangumaran G, Stratton G, Ravichandran S, Shukla PS,Potin P, Asiedu S, Prithviraj B (2017). Microbial degradation of lobster shells to extract chitin derivatives for plant disease management. Front. Microbiol. 8:781-787.

Karunya KS, Reetha D, Saranraj P.Milton, JD. Optimization and Purification of Chitinase Produced by Bacillus subtilis and Its Antifungal Activity against Plant Pathogens. Intl.J.Pharma and Biol.Archives 2011; 2:1680 – 1685.

Kitamura E and Kamei Y. Molecular cloning, sequencing and expression of the gene encoding a novel chitinase A from a marine bacterium, Pseudomonas sp. PE2 and its domain structure. Appl.Microbiol.Biotechnol. 2003;61: 140-149.

Kubota T, Miyamoto K, Yasuda M, Inamori Y, Tsujibo H. (Molecular characterisation of an Intracellular β-N-Acetyl glucosaminidase involved in the Chitin Degradation System of Streptomyces thermoviolaceus OPC-520). Biosci. Biotehnol. Biochem, 2014; 68(6): 1306-1314.

Kuddus SM, Ahmad RIZ.Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. J Genet Eng Biotechnol, 2013;11:39-46.

Kumar A, Singh S (2013). Directed evolution. Tailoring biocatalysts for industrial applications. Crit. Rev.Biotechnol.33(4):365-378.

Kumaran S, Deivasigamani B, Vairagkar U, Balamurugan S, Sakthivel M (2012). Evaluation of chitinase producing and antimicrobial properties of Streptomyces isolated from shrimp shell disposable area. Asian Pac. J.Trop.Biomed.2(2):861-864.

Mathivanan N, Kabilan V, Murugesan K, Purification, characterization and anti-fungal activity of chitinase from Fusarium chlamydasporum, a mycoparasite to groundnut rust, Puccinia arachidis.Can.J.Microbiol. 1998; 44:646 -651.

Mohanta YK (2014). Isolation of cellulose degrading actinomycetes and evaluation of their cellulolytic potential. Bioeng. Biosci. 2(1):1-5.

Nawani NN, Kapadnis BP.(2004).Production dynamics and characterization of chitinolytic system of Streptomyces sp.NK 1057, a well equipped chitin degrader. World J. Microbiol.Biotechnol.20:487-494.

Payal Das, Prateek Kumar, Munendra Kumar, Renu Solanki and Monisha Khanna Kapur*, African Journal of Microbiol.Research. Vol. 11(27). pp.1086-1102, 2017.

Purwani EY, Maggy TS, Yaya R, Jae KH, Yu RP. Characteristics of thermostable chitinase enzymes from the Indonesian Bacillus sp.13.26. Enzyme Microb.Technol.2004;35:147 – 153.

Reissig JL, Strominger JL, Leloir LF, (1995). A modified colorimetric method for the estimation of N-acetylamino sugars. J. Biol. Chem.217:959-966.

Santhi R (2016). Isolation of chitinase producing Streptomyces albus FS12, Production and optimization of extracellular chitinase. Int.J.Adv.Res.Biol.Sci. 3(4): 229-237.

Shanmugaiah V, Mathivanan N, Balasubramanian N, Manoharan P. Optimization of cultural conditions for production of chitinase by Bacillus laterosporus isolated from rice rhizosphere soil. Afr.J.Biotechnol. 2008; 7:2562- 2568.

Singh G, Sharma JR, Hoondal GS 2008. Chitinase production by Serratia marcescens GG5. Asian J. Microbiol. Biotechnol.Environ.Sci. 32:231-236.

Sowmya B, Gomathi D, Kalaiselvi M, Kumar GR, Arulraj C, Uma CS, (2012). Production and purification of chitinase by Streptomyces sp.from soil. J.Adv.Sci.Res.3(3):25-29.

Taechowisan T, Peberdy JF, Lumyong S. Chitinase production by endophytic Streptomyces aureofaciens CMU AC 130 and its antagonism against phytopathogenic fungi. Annal.Microbiol.2003:53: 447-461.

Tasharro I NB, Adrangie S, Fazelia M, Rastegarc H, Khoshayand, Faramarzi MA. Optimization of Chitinase Production by Bacillus pumilus using Plackett – Burman Design and Response Surface Methodology. Iranian J. Pharmaceutical Research 2011;10:759 -768.

Thiagarajan V, Revathia R, Aparanjinib K, (Extracellular chitinase production by Sterptomyces sp.PTK 19 in submerged fermentation and its lytic activity on Fusarium oxysporum PTK2 cell wall). Int. J.curr.Sci, 2011; 1:30-44.

Tsujibo H,Kubota T, Yamamoto M, Miyamoto K, Inamori Y. Characterization of chitinase genes from an alkaliphilic actinomycete, Nocardiopsis prasina opc -13).Appl Environ Microbiol, 2003; 69(2):894-900.

Tweddell, R. J., Jabaji-Hare, S. H., & Charest, P. M. (1994). Production of chitinases and β-1, 3-glucanases by Stachybotrys elegans, a mycoparasite of Rhizoctonia solani. Appl. Environ. Microbiol., 60(2), 489-495.

Vaidya, R. J., Shah, I. M., Vyas, P. R., & Chhatpar, H. S. (2001). Production of chitinase and its optimization from a novel isolate Alcaligenes xylosoxydans, potential in antifungal biocontrol. World Journal of Microbiology and Biotechnology, *17*(7), 691-696.

Wang SL, Chang WT. Purification and characterization of two bifunctional chitinase/lysozymes extracellularly produced by Pseudomonas aeruginosaK-187 in a shrimp and crab shell powder medium. Appl.Environ.Microbiol.1997:63: 380 – 386.

Wilts H, Von Gries N, Bahn Walkowiak B (2016). From waste management to resource efficiency- The need for policy mixes.Sustainability 8:622.

Yassien MAM, Jiman Fatani AAM, Asfour HZ (2014). Production, purification and characterization of cellulose from Streptomyces sp. Afr. J.Microbiol.Res.4:348-354.