Advances in Bioresearch
Adv. Biores., Vol 12 (5) September 2021: 78-84

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.5.7884

Advances in Bioresearch

ORIGINAL ARTICLE

Production , Optimization and Partial Purification of Chitinase by *Pantoea dispersa* isolated from Vegetable dumping site of Patan District.

Dipika Pandya[1*], Dave Dhruv [1], Asha Thakkar [1] and S. A. Bhatt[1]
1. Department of Life Science, Hemchandracharya North Gujarat University, Patan, India
*Corresponding author: dp291187@gmail.com

ABSTRACT
To enhance chitinase synthesis, growth environment parameters were optimized. Chitinase production in liquid medium containing 1.5 percent acid swollen chitin was found to be the best medium for chitinase production. Maximum chitinase production was obtained at 35 ºC, pH 7, during of 96 hrs incubation and at 150 rpm. From Pantoea dispersa extracellular chitinase from Pantoea dispersa was partially purified using ammonium sulphate precipitation followed by concentration using various sizes of concentration tubes namely-40% to 80%. Chitinase seemed to have the highest specific activity at the 50% ammonium sulphate precipitation level, with 1.87 specific activity recorded at this concentration. The results obtained for optimization of production and partial purification are discussed.
Keywords: Pantoea dispersa, chitinase production, Chitin

Received 03.12.2020 Revised 09.08.2021 Accepted 13.08.2021

How to cite this article:
D Pandya, D Dhruv, A Thakkar and S. A. Bhatt. Production , Optimization and Partial Purification of Chitinase by *Pantoea dispersa* isolated from Vegetable dumping site of Patan District.. Adv. Biores. Vol 12 [5] September 2021. 78-84

INTRODUCTION

Chitin is a linear polysaccharide composed of N-Acetyl-D-glucosamine (NAG) units that seem to be 1,4-linked. It is the earth's second most prevalent biopolymer and a consistent source of renewable raw materials [1]. Chitinases (EC3.2.1.14) are the enzymes that hydrolyze 1,4 glycosidic bonds which link chitin NAG residues. Chitin bioconversion naturally occurs and is crucial to reduce complex chitin to simpler carbon and nitrogenous compounds that can be used by other microorganisms [10]. Bacteria, fungi, plants, insects, crustaceans, and vertebrates all synthesize these enzymes to fulfill their nutritional requirements. These enzymes play a key role in the morphogenesis of fungi, insects, and crustaceans. They are involved in pathogen defense processes in plants and viz., most likely, vertebrates as well. Chitinases have a wide range of industrial and pharmaceutical applications, including biocontrol of plant pathogenic fungi and insects, synthesis of chitooligosaccharides and waste management.

Chitinases are consequently important in agricultural and medical domains, and they are particularly significant in the seafood business for the breakdown of crab chitinous waste.

Chitinase is synthesized by a diverse range of organisms, including bacteria, fungus, actinomycetes, yeast, plants, protozoans, coelenterates, nematodes, mollusks, arthropods, and humans [2, 15]. Several factors have been reported to influence chitinase production by bacteria, including chitin, yeast extract, ammonium sulphate, trace elements, tween-20, magnesium sulphate, ammonium chloride, potassium nitrate, diammonium hydrogen phosphate, sodium nitrate, l-glutamine, l-asparagine, peptone, and urea. The conventional parametric or One-Factor-At-a-Time (OFAT) approach for choosing carbon and nitrogen sources for chitinase production is described in a large number of reports [5,12].

Microbial chitinases have been linked to parasite protection in fungi, protozoa, and invertebrates, as well as the degradation of fungal pathogen micelles. Chitinase is also engaged in the defensive systems of both plants and vertebrates. Baculoviruses employed in biological control of insect pests generate chitinase

[18] Chitooligosaccharides are plant defensive compounds that have a wide range of applications in medicine. Chitohexose and chitoheptose have anticancer properties.

MATERIAL AND METHODS

Organism and culture conditions

The chitinolytic bacterium was isolated from vegetable dumping site containing ripen and spoiled vegetable wastes, in Patan, Gujarat, and was identified as *P. dispersa.* The bacterium was cultivated on chitin agar medium consisting of (g/l): acid swollen chitin, 5.0; yeast extract, 0.5; $(NH4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3 and KH_2PO_4, 1.36. The pH of the medium was adjusted to 7.2 and sterilized by autoclaving at 121 ◦C for 15 min [10]**.** Acid swollen chitin was prepared by the method of Hackman 1962.

For Chitinase production, a liquid medium containing (g/l): chitin, 15.0; urea, 0.32; $CaCl_2$, 0.10 and $MgSO_4$ $.7H_2O$, 0.08 was used for chitinolytic enzyme production. The pH of the medium was adjusted to 7.2 and sterilized by autoclaving at 121 °C for 15 min. A five percent inoculum of *Pantoea dispersa* (1×10^8 CFU/ml) was added to 50 ml of liquid media in a 250 ml Erlenmeyer flask and incubated for 48 hrs at 35 °C on a rotary shaker (180 rpm). Consequently, crude enzyme obtained by centrifugation of culture broth at 8000 rpm for 20 min and this crude enzyme was determining unit activity.

Vyas and Deshpande's (1989) procedure was used to analyze chitinolytic activity. A reaction mixture containing 1 ml of 0.7 % acid swollen chitin, 1 ml of 0.05 M acetate buffer, pH 5 and 1 ml of enzyme solution was incubated at 50 ^{0}C for 1 h. At 50 ^{0}C , one unit of chitinolytic activity was defined as the quantity of enzyme required to liberate 1 micromole of N Acetyl D-glucosamine equivalent.

Medium Optimization for chitinase production

Effect of pH and Temperature:

The effect of pH on chitinase activity was checked with various pH ranging from 3.0 to 11.0. pH of the medium was set to desired value with the help of 1N HCl or 1% Na_2CO_3. An actively growing culture of *Pantoea dispersa* was inoculated in a medium having a different pH. These inoculated flasks were incubated at 35 ºC. After incubation, enzyme activity was measured at different time intervals. The effect of temperature on chitinase activity was measured by inoculating chitin containing medium with a young culture of *Pantoea dispersa*. After inoculation, these flasks were incubated at various temperatures, viz., 25 °C, 30 °C, 35 °C, 40 °C, 45 °C. After incubation, enzyme activity was measured at different time intervals up to 96 hrs.

Effect of substrate and Salt Concentration

The effect of salt concentration on chitinase activity was measured by inoculating medium having different concentrations of NaCl (0.5%, 1%, 2%, 3%, 4%) with a young culture of *Pantoea dispersa*. After inoculation, these flasks were incubated at 35 °C. After incubation, enzyme activity was measured at different time intervals.

The effect of concentration of chitin on chitinase production for various concentrations of chitin 0.5%, 1%, 2%, 3%, 4% were tested for *Pantoea dispersa*. An actively growing culture of *Pantoea dispersa* was inoculated in this medium and flasks are incubated at 35°C. After incubation, enzyme activity was measured at different time intervals.

Effect of various nitrogen sources

To study the effect of various nitrogen sources, viz., $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, Urea and Peptone on chitinase production with concentrations of 0.5%, 1%, 2% were tested for *Pantoea dispersa***.** After incubation, chitinase activity is measured at different time intervals.

Effect of carbon sources

To check the effect of carbon sources on chitinase activity, various carbon sources such as glucose, sodium succinate, and sodium citrate with concentrations of 0.5%, 1%, and 2% were tested using ***Pantoea dispersa***. After incubation, enzyme activity was measured at different time intervals.

Effect of cation on chitinase

To study the effect of cation on chitinase production, various metals such as Co^{+2}, Ca^{+2}, Mg^{+2}, Mn^{+2} and Zn^{+2} (in their chloride form) with concentrations of 20 ppm to 100 ppm were tested using the isolate. After incubation, chitinase activity was measured at different time intervals.

Partial Purification of Chitinase

The chitinase enzyme produced by *P. dispersa* was partially purified ammonium sulphate precipitation. The bacterium homogenate was precipitated with ammonium sulphate at 40 -80 percent concentrations, and the chitinase activity of the precipitate and supernatant was measured to determine optimum concentration of ammonium sulphate showed the maximum activity. The precipitate was dialyzed against the same buffer after being dissolved in 0.1 M phosphate buffer (pH:7.0) [3]

RESULT AND DISCUSSION

Effect of Carbon sources on chitinase production:

The result of the effect of carbon sources on chitinase production is depicted in Graph 6, which indicates that maximum production of chitinase occurs after 96 hrs of incubation period. In this work, four different carbon sources were used to check their effect on enzyme production, viz., glucose, starch, sodium citrate and sodium succinate. Chitinase production was affected by all the four carbon sources used in the experiment. Maximum production of chitinase continues till the 96 hr incubation period with glucose and then after it is gradually decreased. All the four carbon sources used in experiments showed a similar effect on chitinase production after 96 hrs of incubation period. Chitinase is affected by glucose and their production is increased with glucose compared to other carbon sources used in the experiment. Chitinase production is affected by glucose concentration and at 2% glucose concentration; chitinase production is at its maximum of 28 units at the 2% concentration.

Effect of nitrogen sources on chitinase production:

Graph 5 shows the effect of different nitrogen sources on chitinase production. Among these nitrogen sources, peptone acts as a good nitrogen source for the production of chitinase enzyme and that gave 36.5u activity. Ammonium chloride also affects the production of the enzymes and has similar activity. Chitinase production is not increased by ammonium nitrate and urea.

Effect of sodium chloride on chitinase production:

The result of the effect of sodium chloride on chitinase production is depicted in graph 3. The results indicate that a gradual increase in sodium chloride concentration enhances the production of chitinase. Maximum chitinase production occurred at a 2.0% concentration. Increasing the NaCl concentration beyond 2% adversely affected the enzyme production and little activity was observed at 3% concentration and then after no more activity was recorded.

Effect of pH on chitinase production:

The result of the effect of pH on chitinase production is shown in Graph 2. This indicates that chitinase enzyme is affected by pH. The maximum production of chitinase enzyme is carried out at pH 7. In the acidic range of pH, enzyme production is reduced. In the alkaline range of pH up to pH 9, production of the enzyme continues, but later at higher pH it is reduced. At pH 7.0, chitinase production was 33.5U/mL.

Effect of temperature on chitinase production:

Chitinase is affected by temperature and maximum production is carried out at a 35°C temperature (Graph 1). A chitinase activity of 49U/mL was obtained at a temperature of 35° C. At higher or lower temperatures, chitinase is affected and its production is substantially reduced.

Effect of cations on chitinase production:

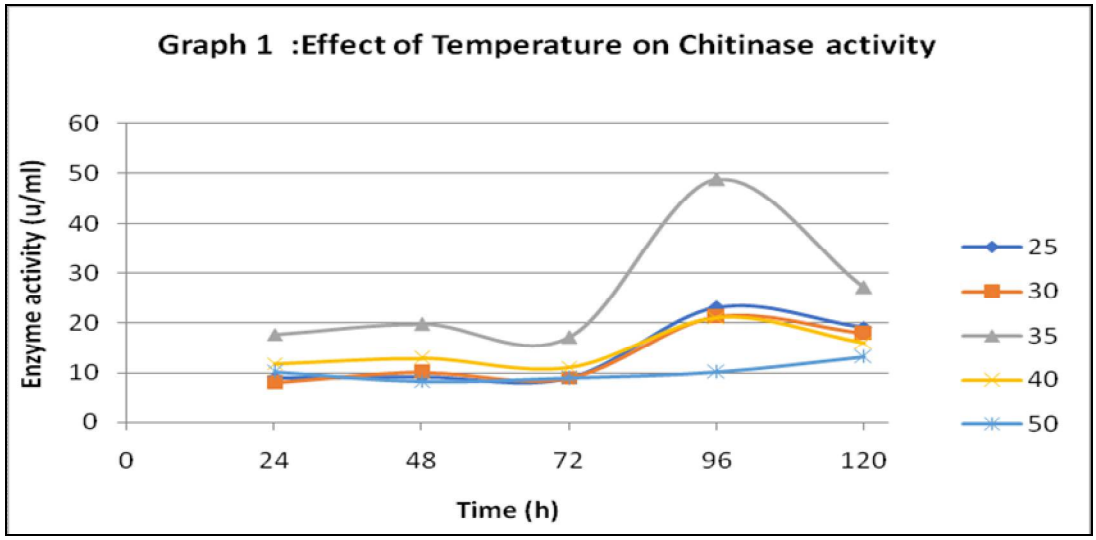
Various cations were used to check their effect on chitinase production, viz., $MnCl_2$, $CaCl_2$, $MgCl_2$, $CoCl_2$ and $ZnCl_2$ (Graph 7 & 8). Manganese chloride and calcium chloride mostly affect the production of chitinase. Other cations have little or no effect on enzyme production used in experiments. Calcium chloride, has given maximum chitinase activity (32.5U/mL).

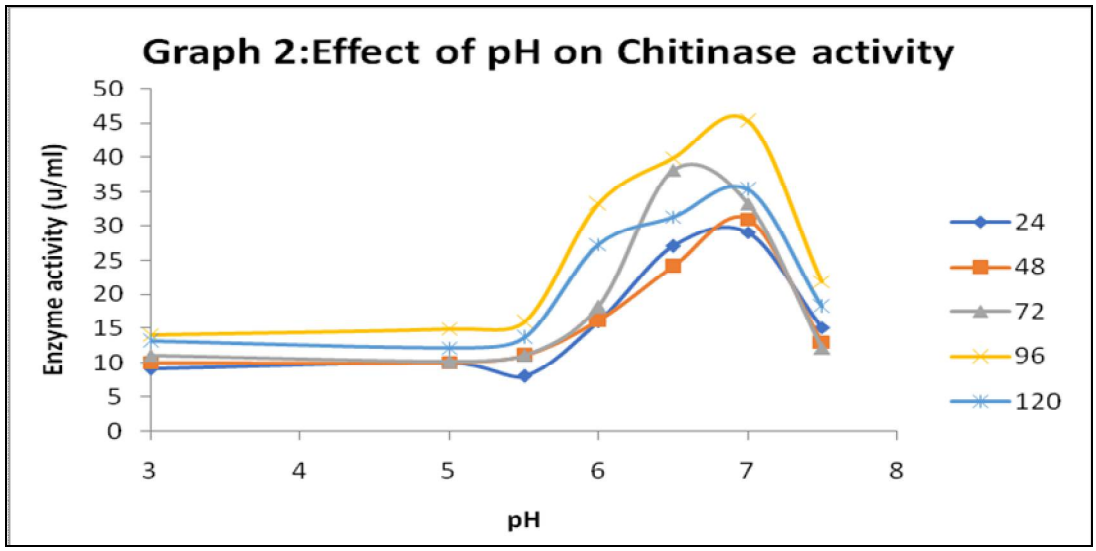
Effect of substrate concentration on chitinase production:

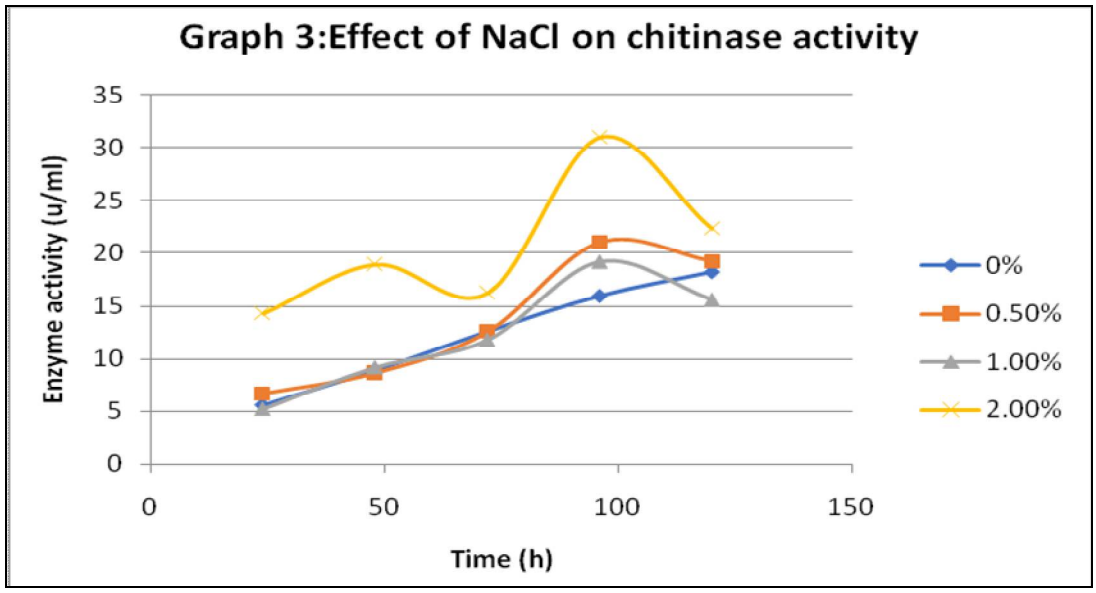
The effect of substrate concentration on chitinase activity is depicted in Graph 4. The results indicate that substrate for the chitinase at a concentration of 3% has given maximum activity after 96 hrs of incubation period.

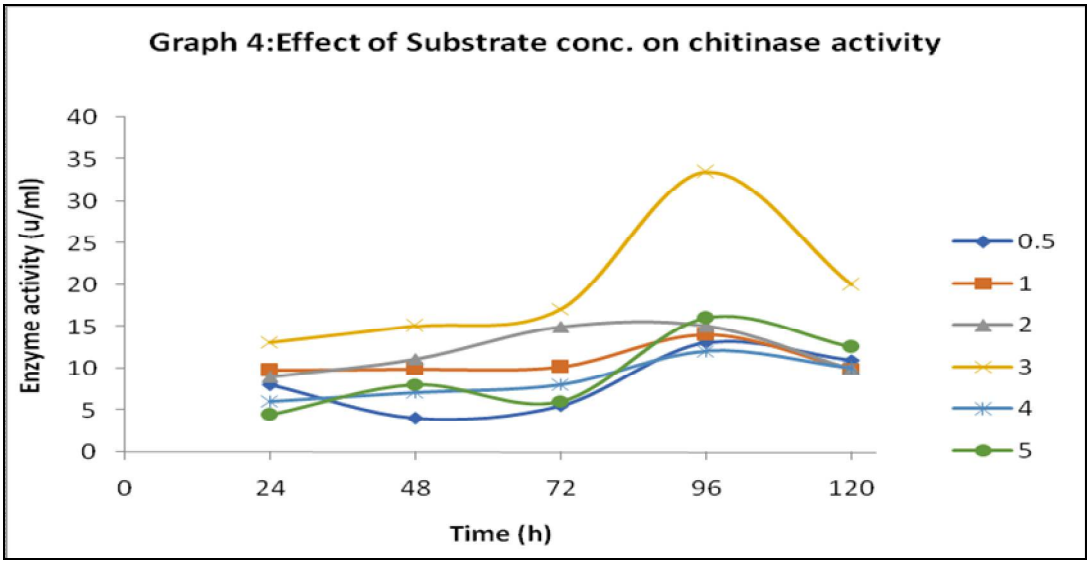
Result of Partial purification of Chitinase

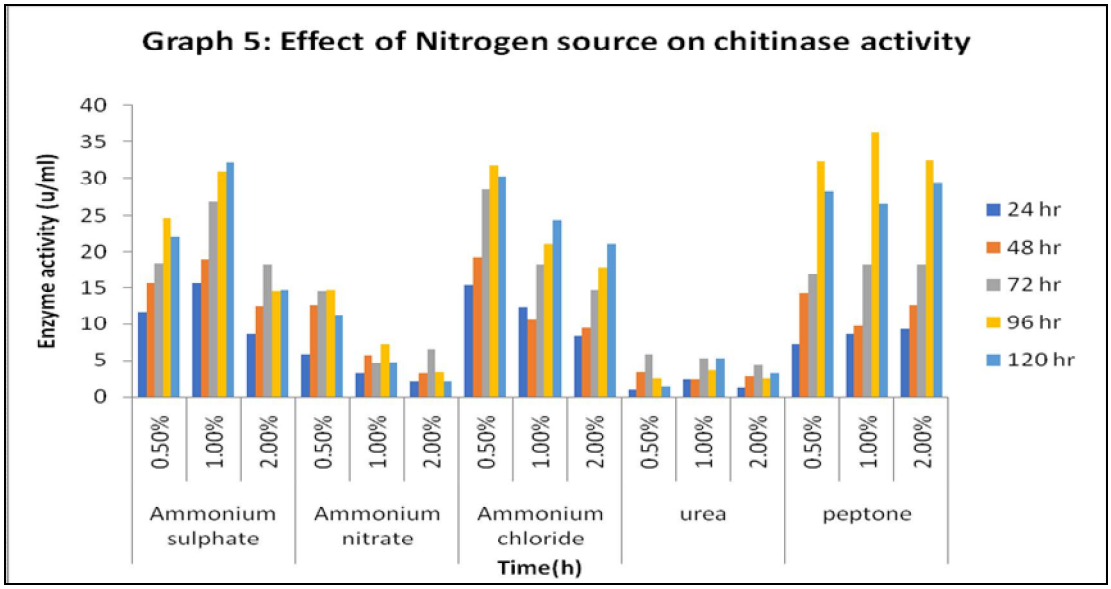
Pantoea dispersa has given highest specific activity of chitinase at the 50% ammonium sulphate precipitation level. Protein precipitation level is gradually increase with the increasing the concentration of ammonium sulphate while total activity of chitinase increased up to the 60% concentration. chitinase 1.87 specific activity was obtained at the 50% concentration.

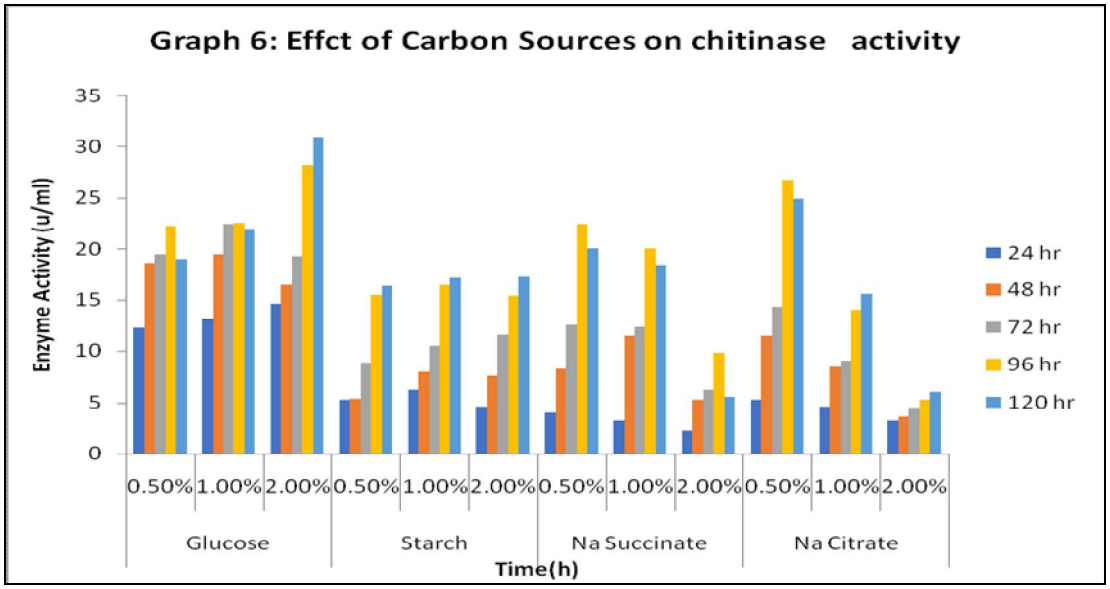


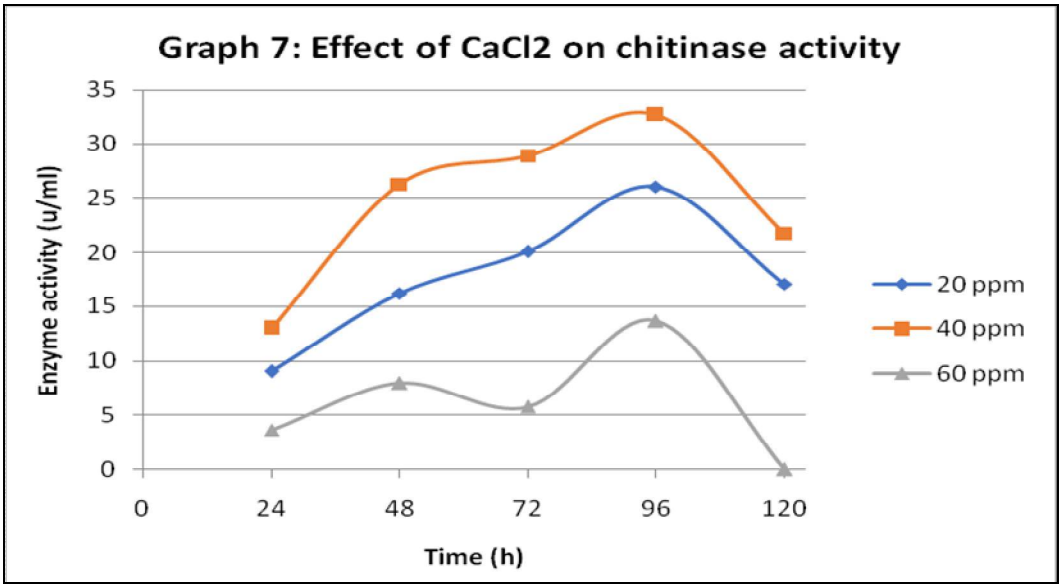












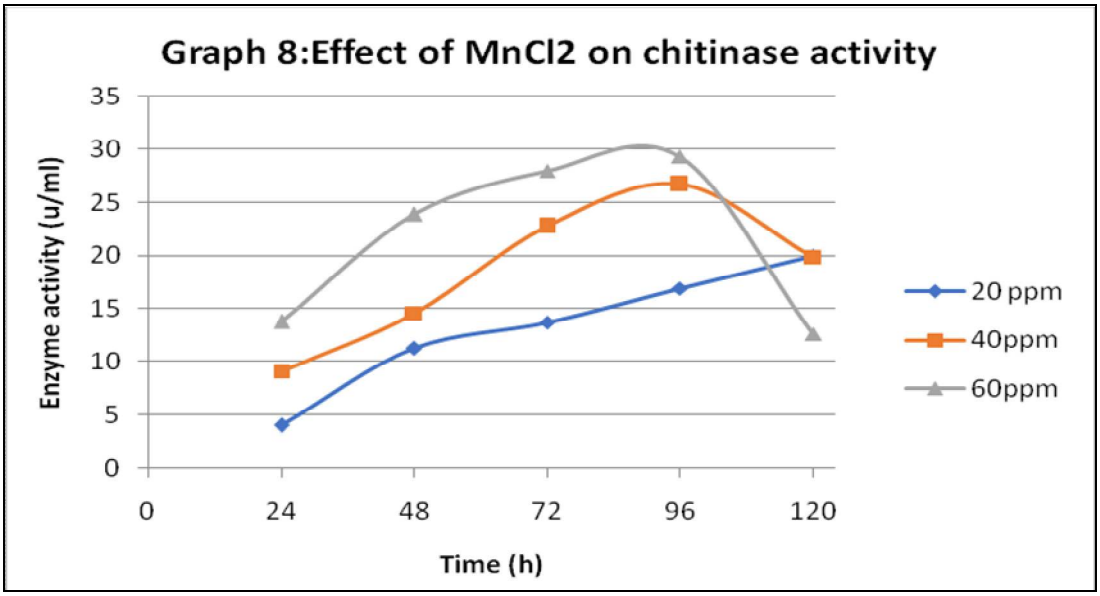


Table:1 Ammonium sulphate precipitation of chitinase from *P. dispersa*

Crude Extract with Ammonium sulphate (Saturation)	Protein (mg/ml)	Unit activity	Specific activity (U/ mg/Protein)
40%	2.9	3.6	1.24
50%	7.8	14.6	1.87
60%	11.6	19.8	1.70
70%	14.8	16.8	1.13
80%	19.3	13.2	0.68

CONCLUSION

Chitinase is affected by various physico- chemical parameter. Along with this chitinase production is also affected by incubation period. Change in the concentration of substrate, carbon, nitrogen sources also enhance the chitinase production. Produced chitinase enzyme from *Pantoea dispersa* has diverse enzymatic activity which is expected to be used in the medical field against fungal infections and in agriculture as biocontrol agents. Purified chitinase from *Pantoea dispersa*, could be an excellent choice in application of food, biotechnology and pharmaceutical industries.

REFERENCES

1. Annamalai, N., Rajeswari, M. V., Vijayalakshmi, S., & Balasubramanian, T. (2011). Purification and characterization of chitinase from Alcaligenes faecalis AU02 by utilizing marine wastes and its antioxidant activity. *Annals of microbiology*, *61*(4), 801-807.
2. Bhushan, B. (2000). Production and characterization of a thermostable chitinase from a new alkalophilic Bacillus sp. BG-11. Journal of applied microbiology, 88(5), 800-808.
3. Demir, A. (2009). SeventekinN."Kitin, Kitosan ve genel kullanım alanları". *Tekstil Teknolojileri Elektronik Dergisi*, *3*(2), 92-103.
4. Faramarzi MA, Fazeli M, Yazdi MT, Adrangi S, Ahmadi KJA, Tasharrofi N, Mohseni FA (2009) Optimization of cultural conditions for production of chitinase by a soil isolate of *Massilia timonae*. Biogeosciences 8:93–99.
5. Felse, P. A., & Panda, T. (1999). Self-directing optimization of parameters for extracellular chitinase production by Trichoderma harzianum in batch mode. Process Biochemistry, 34(6- 7), 563-566.
6. Gohel, V., Chaudhary, T., Vyas, P., & Chhatpar, H. S. (2004). Isolation and identification of marine chitinolytic bacteria and their potential in antifungal biocontrol.
7. Gooday, G. W. (1995). Diversity of roles for chitinases in nature. Chitin and Chitosan, 3, 191-202.
8. Hackman, R. H. (1962). Studies on chitin V. The action of mineral acids on chitin. Australian Journal of Biological Sciences, 15(3), 526-537.
9. Jami Al-Ahmadi K, Tabatabaei Yazdi M, Fathi Najafi M, Shahverdi AR, Faramarzi MA,
10. Zarrini G,Behravan J. (2008) Isolation and characterization of a chitinolytic enzyme producing microorganism, *Paenibacillus chitinolyticus* JK2 from *Iran. Res. J. Microbiol.* 2008;3:395–404.
11. Meena, S., Gothwal, R. K., Mohan, M. K., & Ghosh, P. (2014). Production and purification of a hyperthermostable chitinase from Brevibacillus formosus BISR-1 isolated from the Great Indian Desert soils. Extremophiles, 18(2), 451-462.
12. Monreal, J., & Reese, E. T. (1969). The chitinase of *Serratia marcescens*. Canadian Journal of Microbiology, 15(7), 689-696.
13. Nampoothiri, K. M., Baiju, T. V., Sandhya, C., Sabu, A., Szakacs, G., & Pandey, A. (2004). Process optimization for antifungal chitinase production by *Trichoderma harzianum*. Process Biochemistry, 39(11), 1583-1590.
14. Nawani, N. N., & Kapadnis, B. P. (2005). Optimization of chitinase production using statistics based experimental designs. Process Biochemistry, 40(2), 651-660.
15. Patil, R. S., Ghormade, V., & Deshpande, M. V. (2000). Chitinolytic enzymes: an exploration. Enzyme and microbial technology, 26(7), 473-483.
16. Tan, S. H., Degnan, B. M., & Lehnert, S. A. (2000). The Penaeus monodon chitinase gene is differentially expressed in the hepatopancreas during the molt cycle. Marine Biotechnology, 2(2), 126-135
17. Vaidya, R., Vyas, P., & Chhatpar, H. S. (2003). Statistical optimization of medium components for the production of chitinase by *Alcaligenes xylosoxydans*. Enzyme and Microbial Technology, 33(1), 92-96.
18. VYAS, P., & DESHPANDE, M. V. (1989). Chitinase production by *Myrothecium verrucaria* and its significance for fungal mycelia degradation. The Journal of General and Applied Microbiology, 35(5), 343-350.
19. Wen, C. M., Tseng, C. S., Cheng, C. Y., & Li, Y. K. (2002). Purification, characterization and cloning of a chitinase from Bacillus sp. NCTU2. Biotechnology and Applied Biochemistry, 35(3), 213-219.