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CHARACTERIZATION OF ACIDIC CHITINASE FROM NEISSERIA SPECIES

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A B S T R A C T

Isolated Neisseria species showed highest Chitinase hydrolysis activity on CAM therefore selected for solid state fermentation at 25℃. The extracted crude chitinase enzyme further used for the Chitinase activity by DNS method. The crude Chitinase were characterized by using different parameters such as temperature ranges from 25-50°c (optima-26°c), pH ranges from 5-9 (optima-pH 6.0), substrate concentration 0.2 % – 1.4 % (optima- 0.4 %), metal ions (inhibited by Kcl and increased by $FeSO_4$).

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Introduction

Chitin (the Greek word for "envelope") is one of the most predominant polysaccharide in nature. After cellulose, it is on the second place in biological turnover and it is an important component of many organisms from different taxonomic groups (Gooday *et al*., 1990). Chitin is well-known as an insoluble structural polysaccharide that occurs in the exoskeleton and gut linings of many insects, invertebrates such as crustaceans, protozoa, fungi and diatoms which could be hydrolysed by chitin degrading enzymes such as chitinases (Kramer *et al*., 1986).

Chitin, a linear β-1, 4-N-acetylglucosamine polysaccharide is the most abundant renewable natural resource after cellulose (Deshpande *et al*., 1986). Approximately 75% of the total weight of shellfish, such as shrimp, crabs and krill are considered as waste, and comprises 20 - 58% of the dry weight of the said waste (Wang *et al*., 1997). Biocontrol of pathogenic fungi depends upon antibiosis, competition and lysis (Nandakumar *et al*., 2007).

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Chitinase (EC 3.2.11.14) are groups of Hydrolases enzyme is found in a variety of organisms, including viruses, bacteria, fungi, insects, higher plants and animals and play important physiological roles depending on their origin (Maria *et al.;* 2014). The Chitinases producing organisms have been isolated from a number of sources such as air, water, soil, marine water, etc. (Wang *et al*., 2010; Annamalai *et al*., 2010) Chitinases have been isolated from the stomach of certain mammals including humans. Although mammals do not produce chitin; they have two functional Chitinases – chitoriosidase – CHT1 and acidic mammalian chitinase – AM Case that high sequences similarity, but lack chitinase activity (Gooday *et al.*, 1990). The Chitinases of the family 18 have been found to possess a common (α/β) 8– barrel domain consisting of 8 α- helices and 8 β- strands. They are distributed in a wide range of organisms, including bacteria, fungi, plants, insects, mammals and viruses (Cody *et al.*, 1990).

A wide variety of Microorganisms live in nature at various temperatures for their survival. They not only survive at high temperature but also carried out their activities according to maintained conditions. Many enzymes such as chitinases, amylase, protease, lipases, Xylanases etc. are industrially important.

The present study is carried out in the investigation of novel chitinases from bacteria under standard and maintained conditions by offering following materials and methods.

2. Methods and Materialss

2.1 Solid state fermentation:

Solid-state fermentation was employed for production of chitinase. 10 g of Rice bran was transferred to the seven individual 250 ml cotton plugged Erlenmeyer flasks. The flasks were autoclaved at 15 lb/inch2 pressure and 121ºC for 15 min., and cooled the medium at room temperature. The chitinase positive were inoculated in SSF and incubated for 14 days for chitinase production.

2.2 Enzyme Extraction

After 7 days of incubation period, 30 ml of 0.05 M Phosphate buffer (pH 6.0) was added to the fermented substrate in first flask. The contents of the flask were crushed with the help of a glass rod and flasks were rotated on a rotary shaker at 120 rpm for 1 h at 30ºC afterwards the fermented medium filtered through whatman filter no. 1 filter paper. After filtration, contents were centrifuged at 5,000 rpm for 10 min at 4ºC and clear supernatant from each of the tubes was collected for further studies and tubes was stored at 4°C until used. This whole procedure was repeated for remaining six SSF flasks.

2.3 Chitinase Enzyme activity

Chitinase enzyme activity was assayed by using the DNS method to measure the amount of reducing sugar liberated from chitin. The crude enzyme protein was used for measuring the activity at pH 6 (phosphate buffer, 0.5) at 37^0c for 30 min.

2.4 Chitinase enzyme unit activity

According to the International Union of Biochemistry 1 enzyme International unit has been defined as the amount of enzyme required to release 1µmol of reducing sugar in 1 min. at 40°c and at atmospheric pressure.

2.5 Characterization of crude chitinase

The effect of some factors that influence crude chitinase activity in the reaction mixture at different pH, temperature, substrates concentration and metal ions was studied.

2.6 Effect of Temperature on chitinase activity

The effect of temperature on the chitinase activity was determined at pH 6 with different temperature ranges from 25-55°C under standard assay conditions and optimum temp. Were determined by using DNS method.

2.7 Effect of pH on the chitinase activity

The effect of pH on the chitinase activity was determined at 28°c with different pH values (5 to 9) by using standard phosphate buffer solutions and optimum pH were determined by using DNS method.

2.8 Effect of metal ions on chitinase activity

The effect of various metal ions on chitinase activity was determined at pH 6 and 37°c by using different metal ions such as FeSo4, KI, CaCl2, MnSo4, Kcl, CoCl2, $CuCl_2$. Chitinase assay was then performed by using DNS method.

2.9 Effect of Substrate concentration on chitinase activity.

The effect of Substrate Concentration on chitinase activity was determined at pH 6 and 37°c by using different concentrations of substrate: 0.1%, 0.25%, 0.5%, 0.75%, 1%, 1.5% and 2% w/v of colloidal chitin

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and optimum substrate concentration were determined by using DNS method.

3. Results

3.1 Effect of temperature on chitinase activity

It seems that 25°C is the highest activity of crude chitinase while at the other conditions activity is decreased. However, it retains about 90 % activity at 25°c and 35 % at 50°c at pH 6.0 therefore the activity decreases sharply as the temperature increases.

Table 3.1 Effect of temperature on chitinase activity

Sr. No	Temperature (°C)	Chitinase activity (µg/ml/min)
1	25	9.65
2	35	8.14
3	45	6.15
4	55	4.12

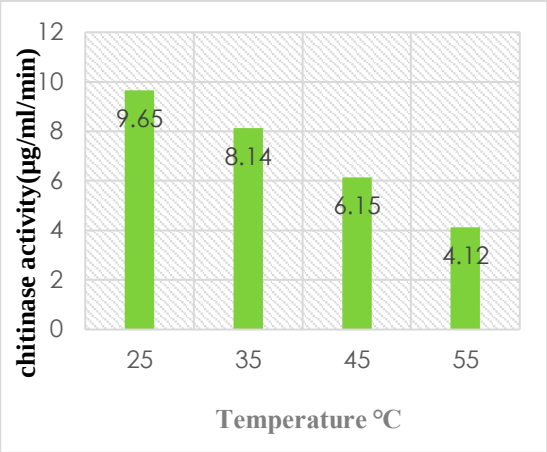


Figure 3.1 Effect of temperature on chitinase activity

3.2 Effect of pH on chitinase activity

It seems that, at pH value 6 optimum chitinase production obtained as compared to pH 5, 7, 8 and 9. Table II and graph II indicate that 6 is the optimum pH for chitinase activity.

Table 3.2 Effect of pH on chitinase activity

Sr. No	**pH**	Chitinase activity (µg/ml/min)
1	5	3.61
2	6	6.11
3	7	5.62
4	8	4.12
5	9	2.10

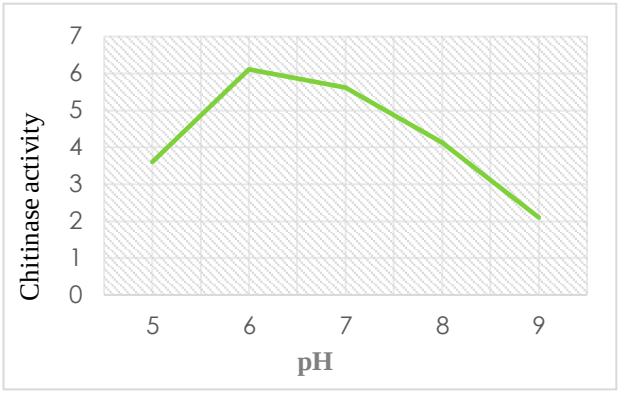
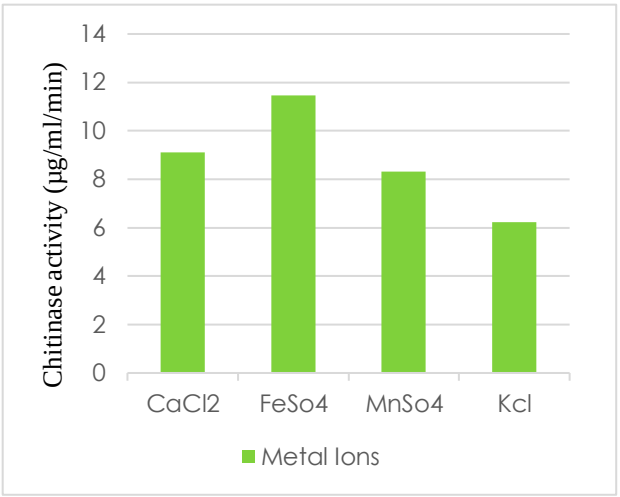


Figure 3.2 **Effect of pH on chitinase activity**

3.3 Effect of Metal Ions on Chitinase activity

Sr. No	Metal Ions	Chitinase activity (µg/ml/min)
1	CaCl2	9.10
2	FeSo4	11.46
3	MnSo4	8.32
4	Kcl	6.22

The chitinases enzyme activity slightly increases after the addition of $FeSO_4$ while by the addition of Kcl and $Cacl_2$ chitinase activity was strongly inhibited



It seems that, the effect of heavy metals on activity of crude chitinase was obtained by the addition of Kcl while FeSo4 increases the crude chitinase activity.

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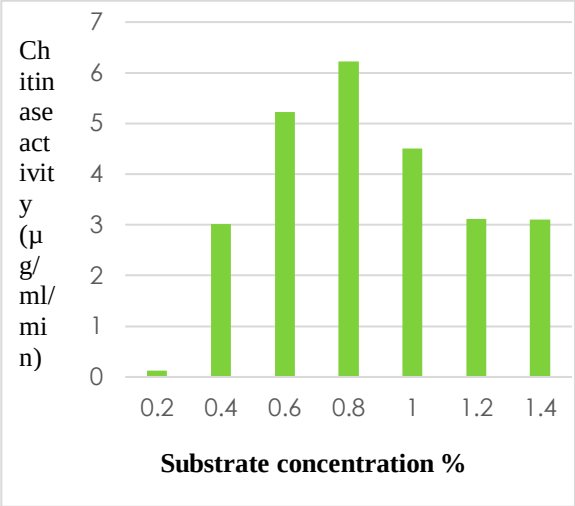
Figure 3.3 Effect of Metal Ions on Chitinase activity

3.4 Effect of Substrate concentration on Chitinase activity

Sr. No	Substrate concentration %	Chitinase activity (µg/ml/min)
1	0.2	0.12
2	0.4	3.02
3	0.6	5.22
4	0.8	6.22
5	1.0	4.51
6	1.2	3.12
7	1.4	3.10



Discussion

Soil bacteria are rare for chitinase production. In the present study, in the Secondary screening chitinase positive *Neisseria* species were confirmed by solid state fermentation in rice bran. Solvent extraction method employed for crude chitinase extraction.

The Crude chitinase were characterized at different pH (5-9), temp (25-50^{0}c), substrate concentration (0.2 - 2.0%) , heavy metal ions , incubation time as like explained by Mathur *et al*., 2011: Deshpande *et al*.,1986, Dhar *et al*., 2010, Gooday *et al*., 1990.

According to Tsukmto *et al*., 1984: shgui *et al*., 2005, Chitinase generally shows enzyme activity from pH (4-9) and we got optimum chitinase activity at pH 6 and this results were matched with Anuradha *et al.*, 2013 because she has got optimum chitinase activity at pH 6. Kim *et al* 2002 has got optimum enzyme activity at 30^{0}c & Buckley et .al 2007 has got optimum enzyme activity at 28°c temperature while our present optimum chitinase activity were observed at 28^{0}c. Gooday *et al.*,1990 has got optimum 0.6% substrate concentration for chitinase activity while Our present chitinase shows optimum enzyme activity at 0.8% of colloidal chitin as a substrate and this results were also matched with Uma *et al.*, 2012 : Planiet *et al.*,2014. The present chitinase were strongly inhibited by Kcl & strongly activated by FeSo4 and these results were matched with yong *et al.*, 2005; Shigui *et al*., 2015: Bajekal *et al*., 2006.

Conclusion

The production of chitinases widely applied in the field of chemistry, biomedical, biotechnological, agricultural and environmental protection. Wide scope for extensive research to achieve industrial scale production from the chitinases by Solid State Fermentation.

The chitinase from *Neisseria* species was active between pH 5 to 9 with optimum pH 6.0 and the chitinase activity was 6.03µg/ml/min. beyond the pH 6.0 chitinase lost its activity. The chitinase was most active between temperatures 25°c to 50°c. The optimum chitinase activity at 25°c was 9.65µg/ml/min. The chitinase was most active between substrate concentrations 0.2 to 1.4. The optimum chitinases activity was 6.22 µg/ml/min. at 0.8% substrate concentration.

The chitinases enzyme activity slightly increases after the addition of $FeSO_4$ while by the addition of Kcl and $Cacl_2$ chitinase activity was strongly inhibited. Therefore present chitinase is novel in nature.

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