Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2011 Vol. 1 (3) July-September, pp. 161-165/Loni and Bajekal

Research Article

Alkaline Chitinase from *Bacillus firmus* SBPL-05 Isolated from Alkaline-Saline Environment of Lonar Lake

***Prasad P Loni and Shyam S Bajekal**
Department of Microbiology, Yashwantrao Chavan college of Science Karad
Vidyanagar Karad-415124
**Author for Correspondence*

ABSTRACT

Chitinases are the enzymes that break down the second most abundant polymer chitin. Chitinase produced from the microorganisms from extreme environment are considered to have great potential with numerous uses. In this paper we report the characters of the chitinase from *Bacillus firmus* strain SBPL-05 isolated from alkaline-saline environment of Lonar Lake. The enzyme had maximum activity at pH-10 and temperature optima of 37^0C. It also showed typical response to the substrate concentration and activity even at 3% salt concentration. The isolate was identified as *Bacillus firmus* by biochemical tests as well as 16S r RNA analysis. The enzyme obtained from SBPL-05 isolated from such extreme environment could have great application in biocontrol and sea food waste management.

Key Words: Chitinase, Lonar Lake, Biocontrol

INTRODUCTION

Chitinases are the group of enzymes that decompose chitin, a linear β-1-4 linked N- acetylglucosamine and either belong to family 18 or family 19 of glycosyl hydrolases (Davies *et al.,* 1995). Chitinases occur in a wide range of microorganisms such as bacteria, fungi and actinomycetes. Such chitinases obtained from the microorganisms has wide use in various fields such as medicine, agriculture and even in cosmetics. The Chitinolytic microorganisms have been widely used as biological control agent as the chitinases are involved in the antagonistic activity against many phytopathogenic fungi (Gohel *et al.,* 2006). The chitinases being an antifungal protein has a great biotechnological interest because of their potential use as food and seed preservative agents and for engineering plants for resistance to phytopathogenic fungal protoplasts (Dempsey *et al.,* 1998).

The sea food industry is also a major source of chitinous waste. The recycling of such waste is very important to maintain the carbon, nitrogen balance in the ecosystem. This process of recycling can be achieved by the chitinolytic microorganisms as well as their enzymes (Mukherjee *et al.,* 1997). Bacteria produce chitinases mainly to degrade chitin and utilize it as an energy source (Hoster *et al.,* 2005).

Proper application of such chitinases in various fields requires a proper understanding of its properties. Here we report the studies on chitinase from *Bacillus firmus* isolated from the saline –alkaline Lonar lake. The study includes isolation of the bacteria and its characterization. The report also includes production, purification and characterization of the alkaline chitinase obtained from *Bacillus firmus*.

The Lonar lake is situated in the Buldhana district of Maharashtra. The peculiar property of this lake is that the water of the lake as well as its littoral soil has high alkaline pH of 9.0 to 10.5. The microorganisms living in such environments would be of extreme species and also having unique feature with respect to their biochemical activities as well as their enzymes (Loni *et al.,* 2009).

MATERIALS AND METHODS

Isolation of the Bacterium

Soil samples were collected from the littoral zone of the lake in selected sites. Isolation of the chitinolytic microorganisms from the soil sample was done on chitin agar medium consisting colloidal chitin as a sole source of carbon. The colloidal chitin was prepared from practical grade crab shell chitin (Loba chem.) as described by Hsu and Lockwood (1975). The medium consisted of the following composition (g/l):-Colloidal chitin (8.0), MgSO47H2O (0.5), K2HPO4 (0.7), KH2PO4 (0.3), FeSO47H2O (0.01), MnCl2 (0.001), NaCl (0.03) and Agar (20), pH-10.5. The same medium was used for maintaining the pure culture. The plates were incubated at 37^0C for 3 days. After incubation, the colonies showing large zone of clearance (CZ) indicating chitinolysis were selected (Bansode *et al.,* 2006)

Research Article

Chitinase production

Chitinase enzyme production from the selected potent isolate was done in the colloidal chitin broth with pH-10.5. The sterilized medium was inoculated with 1% of spore suspension (0.1 O.D at 600 nm) of the selected isolate. The inoculated culture was incubated at 37^0 C on rotary shaker at 150 rpm. The chitinase production was analysed at every 24hr intervals. The fermented culture broth was centrifuged at 8000 rpm for 10 min to remove the cell mass. The cell free supernatant was used for the enzyme extraction by 40% to 60% ammonium sulphate. The precipitate obtained by centrifugation at 5000 rpm for 10 min. was dissolved in phosphate buffer (pH-10) followed by dialysis. This partially purified enzyme was preserved in plastic bottles at 0^0C and used for further study.

Chitinase assay

The assay system of Monreal and Reese (1969) estimating reducing sugars released by the enzyme action was adopted for chitinase assay study. In 1ml buffer (pH-10), 0.5ml of substrate was added followed by 0.5ml of purified enzyme. The tubes were incubated at 37^0C in water bath for 1hr. Then the reaction was arrested with 2ml of Dinitro Salicylic Acid (DNSA) followed by heating in water bath for 10 min. The coloured solution was centrifuged at 5000 rpm for 5 min and absorbance of the supernatant was measured at 540 nm wavelength. The reducing sugar was estimated from the glucose curve. One unit of the enzyme is defined as the amount of enzyme which catalyses the release of 1µm of reducing sugar per minute under assay condition.

Taxonomical study

The isolated potent chitinolytic bacterium was studied for morphological and physiological characters with basic biochemical tests. The isolate was finally identified up to species level by 16s rRNA analysis (Courtesy NCCS, Pune).

Effect of pH on enzyme activit

The effect of pH was tested using four different buffers of (0.05M) strength as Glycine HCL (pH 2.2-3.4), Acetate (pH 3.6-5.6), Phosphate (pH 6.0-8.0) and Glycine NaOH (pH 8.6-11.2). The test was carried out using 0.5ml of enzyme in buffers ranging from 2.2 to 11.2 at 37^0C for 1 hr. After which the residual activity was determined.

Effect of temperature on enzyme activity

The thermal stability of the enzyme was determined by incubating the enzymes in optimum pH buffer and variable temperature range as 0, 10, 28,37,55,80 and 100^0C respectively for 1hr. Then those with high temperature were cooled rapidly and then the residual activity of each were measured.

Effect of substrate concentration on enzyme activity

Varying volumes of 1% substrate i.e. chitin were added in assay systems with fixed volume of enzyme i.e. 0.5ml. The process was carried out at their respective optimum pH and temperature for 1hr before the measurement of residual activity.

Effect of salt (NaCl) concentration on enzyme activity

The effect of salt concentration on enzyme activity was studied at variable NaCl concentration ranging from 0%, 0.5%, 1%, 2% and up to 10%. The varying salt concentration was added to the assay system in fixed volume and then the measurement of residual activity was done.

Statistical analysis

Statistical analysis was done for all the collected data**.** The reported data represents the mean values for all replicated experiments $\pm$ standard error of the mean.

RESULTS AND DISCUSSION

From the collected soil samples several chitinolytic microorganisms were obtained. The isolate named NW-3-2 was selected for the further study due to its high potential in chitinolysis. The colony characters of the isolate are mentioned in Table-1 as well as the morphological and biochemical characters of the isolate are presented in Table-2 and Table-3 respectively. It was identified as *Bacillus firmus* by performing 16s rRNA analysis. The phylogenetic tree is mentioned in figure-1.

Table 1: Colony characters

Size	Shape	Margin	Elevation	Consistency	Opacity	Colour
3-mm	Circular	Regular	Raised	Moist	Opaque	Creamy

Table 2: Morphological characters

Gram Nature	Motility	Spore
Gram Positive single rods	Motile	Central Spore forming cells

Table 3: Biochemical tests

Tests	Results
Catalase	+
Oxidase	+
Casein Hydrolysis	+
Starch Hydrolysis	+
Gelatin Hydrolysis	+
Urea hydrolysis	-
Glucose	+
Lactose	-
Maltose	+
Mannose	-
Mannitol	+
Sucrose	+

On the Chitin agar incubated at 37^0 C the colony of NW-3-2 bacterium was observed with creamy colour, 3-mm in size and circular in shape, the colony had regular margin, raised elevation and moist in nature. The microscopic observation resulted that the cells were Gram positive short rods, motile in nature and spore are formed centrally, which are the typical characters of the *Bacillus* species.

From the certain biochemical tests, it was observed that the strain was positive for catalase and oxidase test, also capable of hydrolyzing casein, starch and also gelatin liquefaction test positive but urea hydrolysis test negative. Except lactose and mannose the bacterium utilized glucose, maltose, mannitol and sucrose. The Phylogenetic tree of the *Bacillus firmus* Strain SBPL- 05 is shown in Fig.1 which is based on 16s rRNA sequence comparisons. Here it can be observed that the Strain SBPL- 05 is closely clustered with members of *Bacillus firmus* Strains. The Gene Bank accession number for *Bacillus firmus* Strain SBPL- 05 is AB650511.

The chitinase enzyme was obtained after 8 days of fermentation where maximum production of enzyme was obtained as 66.02 U/ml. After partial purification the enzyme was subjected for the further study.

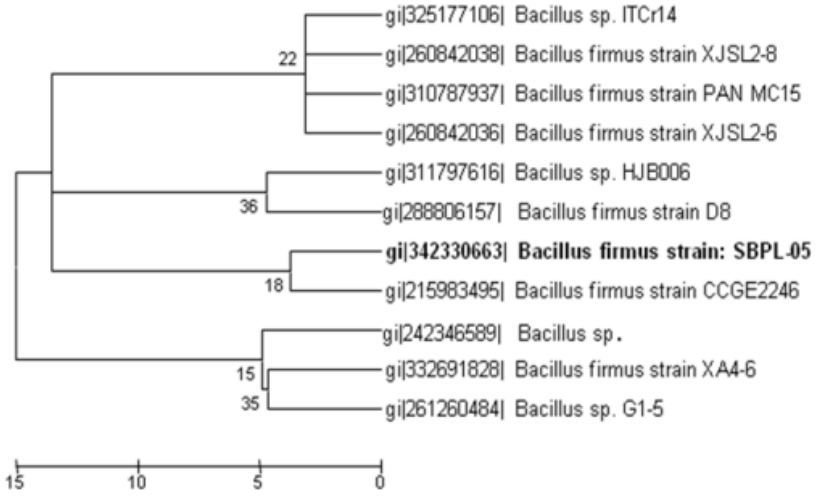

Figure 1: Phylogenetic Tree of *Bacillus firmus* Strain SBPL- 05

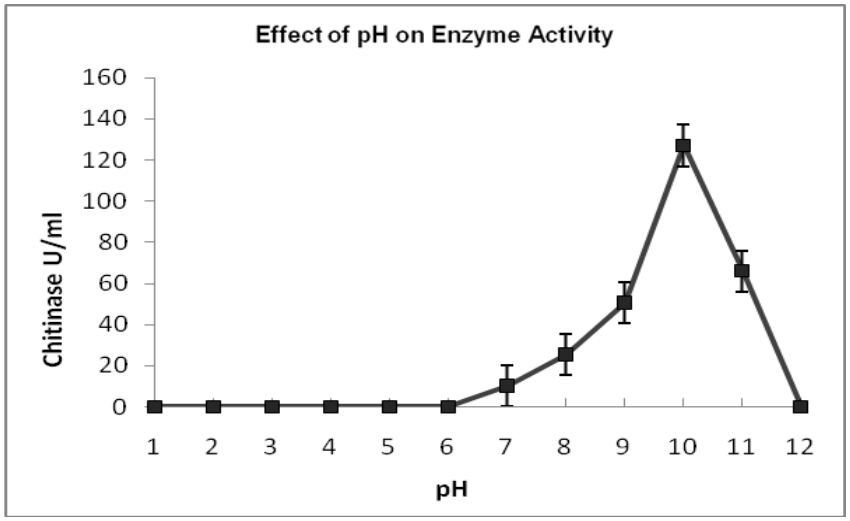

Figure 2: Effect of pH on Enzyme Activity

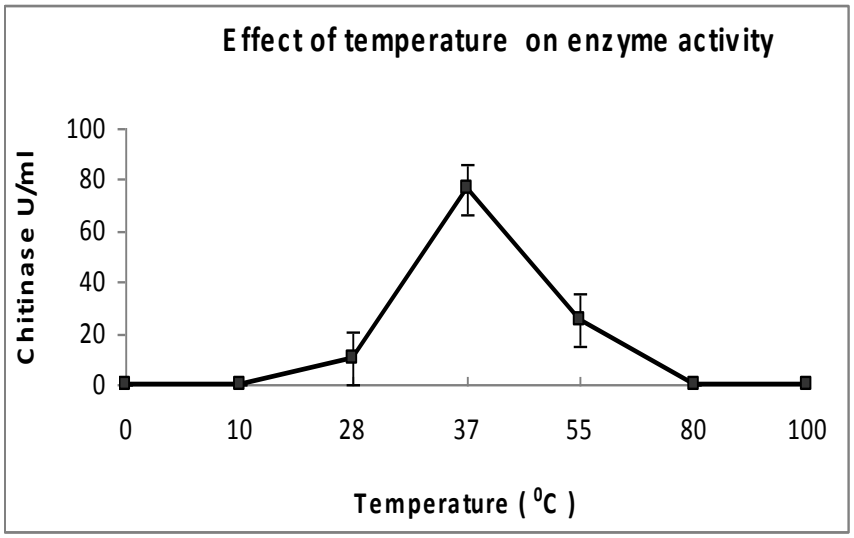

Figure 3: Effect of Temperature on enzyme activity Effect of substrate concentration

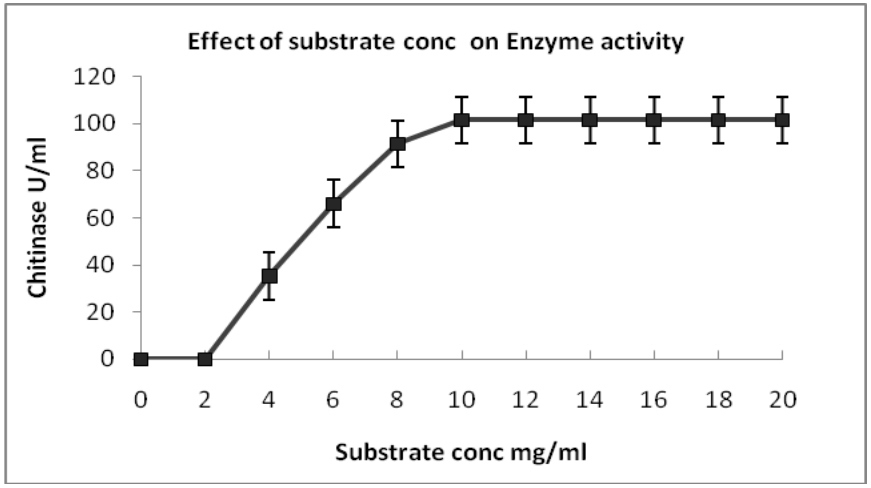

Figure 4: Effect of substrate concentration on Enzyme activity

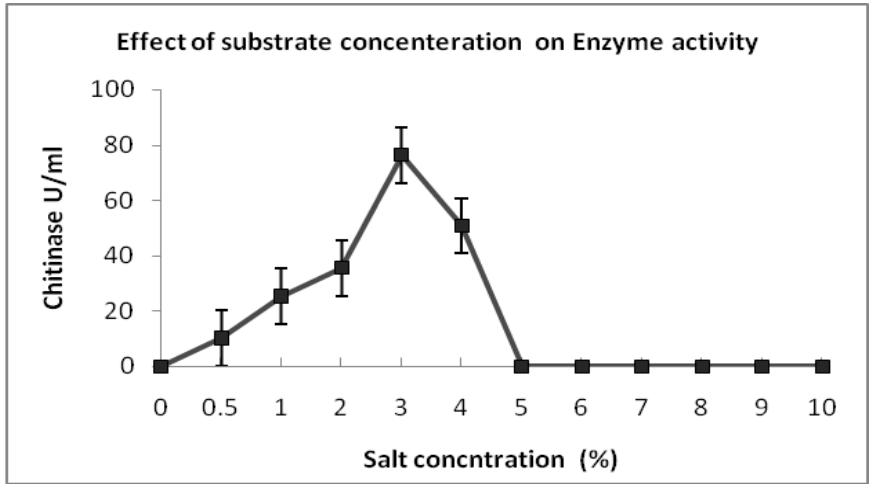

Figure 5: Effect of Salt (NaCl) concentration on Enzyme activity

Effect of pH on enzyme activity:

The effect of pH on chitinase obtained from the *Bacillus firmus* is shown in figure -2. The enzyme activity was shown up to pH-11 and optimum at pH-10 with maximum enzyme production i.e. 126.96 U/ml. This determined the optimum pH of the enzyme which was used for further experiments. Below pH-7 it did not show any activity, it meant that the enzyme had maximum activity in alkaline range.

Effect of temperature on enzyme activity:

The chitinase enzyme stability due to temperature is shown in figure -3. It was observed that the chitinase had the optimum activity at 37^0C temperature with enzyme production of 76.18 U/ml. Least activity was observed at 10^0C, 28^0C and 55^0C and no activity at 0^0C, 80^0C and 100^0C.

The chitinase from *Bacillus firmus* showed a typical relationship with its substrate where it is saturated at low concentration and reach at maximum activity. The observations are noted in figure -4.

Effect of Salt (NaCl) concentration on enzyme activity

As shown in the figure 5, the NaCl played the significant role on enzyme activity. The enzyme showed activity at 0.5%, 1%, 2%, 4% and maximum activity at 3% salt concentration and the no activity at 5% and further.

DISCUSSION

The potent chitinolytic bacteria isolated from alkaline – saline environment was identified as *Bacillus firmus.* Further from the current study it was concluded that the chitinase enzyme obtained from this bacteria is found to have maximum activity at alkaline pH range and also most stable at pH-10. The enzyme has maximum activity at temperature of 37^0C and 3% salt concentration. As it was observed the typical relation with substrate concentration, which is an indicative of good catalytic efficiency.

Thus the chitinase with alkaline in nature is considered to be useful in seafood waste management for the chitinous waste as well as in biocontrol. So the chitinase obtained from *Bacillus firmus* can be effectively applied in the field of waste treatment and which requires the further studies.

AKNOWLEDGEMENT

The authors are thankful to the NCCS (Pune).Also to the management and administration of Yashwantrao Chavan college of Science Karad for providing the necessary facilities in Department of Microbiology for this work.

REFERENCES

Bansode VB, Bajekal SS (2006). Characterization of chitinases from microorganisms isolated from Lonar Lake, *Indian Journal of Biotechnology* **5** 357-363.

Davies, **G**. and **Henrissat**, **B**. (**1995**) Structures and mechanisms of glycosyl hydrolases. Structure **3** 853-859.

Dempsey DA, Silva H, Klessig DF (1998). Engineering disease and pest resistance in plants. *Trends in Microbiology* **6** 54-61.

Gohel V, Singh A, Misuria V, Phadnis A and Chhatpar HS (2005) Bioprospecting and antifungal potential of chitinolytic microorganisms. *African Journal of Biotechnology* **5** (2) 54-72.

Hoster F, Schmitz JE and Daniel R (2005). Enrichment of Chitinolytic microorganisms: isolation and characterization of a chitinase exhibiting antifungal against phytopathogenic fungi from Novel Streptomyces strain. *Applied Microbiology and Biotechnology*. **66** 434-442

Hsu SC and Lockwood JL (1975) Powdered chitin agar as a selective medium for enumeration of actinomycetes from water and soil. *Applied Microbiology* **29** 422-426.

Loni PP and Bajekal SS (2009). Chitinase Producing potential of saline-alkaline environment of lonar lake, *Journal of Environmental Research and Development*. **4** (1) 161-164.

Monreal J and Reese ET (1969). The chitinase of *Serrratia marcescens*. *Canadian Journal of Microbiology*. **15** 689-696.

Mukherjee G and Sen S K (2004). Characterization and identification of chitinase producing *Streptomyces Venezuela* P10, *Indian Journal of Biotechnology* **42** 541-544