e-ISSN: 2248-9126
Vol 5 | Issue 3| 2015 |195-198. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

PRODUCTION AND DYNAMICS OF AMYLASE FROM *BACILLUS FLEXUS*

Tambekar DH[1*], Tambekar SD[2] and Mohod NA[1]

[1]Post Graduate Department of Microbiology, S.G.B. Amravati University, Amravati – 444604, Maharashtra, India.
[2]Department of Microbiology, D.B. Science College, Gondia – 441614, Maharashtra, India.

ABSTRACT

Most of the industrial processes are carried out at high temperature and at high pH and need enzyme having high thermo and pH stability. These extremophilic conditions are found in alkaline water bodies like sea or alkaline lakes such as Lonar Lake. The present study deals with isolation, production and dynamics of amylase from bacterial strain isolated from the alkaline Lonar Lake. Isolation of bacteria was done by using Horikoshi medium and screened for production and partial characterizations of amylase. A total of six bacterial cultures were isolated and one isolates was selected for further studies. Isolate was characterized by cultural, morphological and 16S rRNA gene sequencing. The results of 16S rRNA sequencing showed *Bacillus flexus* (DHT13). Amylase from *Bacillus flexus* having optimum temperature 60°C, optimum pH 10, optimum enzyme concentration, optimum substrate concentration and finds potential applications in food, pharmaceutical and detergent industries.

Keywords: Lonar Lake amylase, Haloalkaliphiles, *Bacillus*.

INTRODUCTION

Microbial amylase could be usable recourse in pharmaceutical with the advent of the new knowledge in biotechnology, the spectrum of amylase application has widened in many other field, such as clinical, medical and analytical chemistry as well as their widespread saccharification [1]. Hence alkaline amylase producing bacteria are of great importance in detergent and textile industry due to its high thermo-stability, pH stability. The amylase is most important industrial enzymes, accounting for about 60% of total enzyme market [2, 3]. The uniqueness of Lonar Lake is its salinity and alkalinity which is harbors various unidentified, unique haloalkaliphilic bacterial species which can be produces industrially important enzymes [4]. These enzymes are thermostable, resistance to alkali and most of the denaturing chemicals [5]. Very less study has been done on amylase from bacilli of Lonar Lake which can withstand high temperature as well as high pH and has wide application in different industries. As there is large demand of amylase, isolation and production of amylaseenzyme is most important to fulfill this demand [6]. Therefore, attempt was made to study deals with isolation, production and dynamics of amylase from bacterial strain isolated from the alkaline Lonar Lake.

MATERIALS AND METHODS

Collection, Enrichment, Isolation and Identification of protease producing bacteria

Total 28 samples (sediment and matt) were collected in august2014 from alkaline Lonar Lake. Sample is diluted to 1/100 in sterilized normal saline and heated at 80^0C for 15 min to destroy all the vegetative microbial cells. The suspension was further diluted to 10-7 dilutions one ml each was subcultured and screened for proteolytic activities on starch agar medium at 37^0C for 72 h and observed for zones of clearance, indicating proteolytic activities. The bacterial isolates with prominent zones of clearance on starch agar medium were processed for identifications based on cultural, morphological and biochemical was done by commercially available Hi-media

Corresponding Author:-**Tambekar DH** Email: diliptambekar@rediffmail.com

Rapid detection kit KB003 and KB009. The 16S rRNA gene sequence was performed at Agharkar Research Institute, Pune [7].

Preparation of crude enzyme extracts

The 100 mL Starch nutrient medium was inoculated with culture and incubated for 48 h at 37°C in incubator. After 48 h incubation, centrifuged the broth at 5000 rpm for 15 min. The supernatant served as crude enzyme source.

Characterization and Assay of Amylase

The standard graph of maltose was prepared by adding different concentration of standard maltose (1 mg/mL) and 2mL DNS solution into a series of test tubes and incubated all tubes in boiling water bath for 5 min and then adds 1mL NaKtartarate to stop the reaction. Estimation of amylase was carried out with 2.5 mL of (1%) starch solution; 2.5 mL of PO_4 buffer, 1mL of NaCl and 1mL of enzyme source in a test tube and incubated inboiling water bath for 5 min and then addition of 1mL of NaK^+tartarate to stop the reaction.The effect of pH on alkaline amylase was determined by assaying the enzyme activity at different pH ranging from 7 to 11, effect of temperature by incubating from 30°C to 80°C using the PO_4 buffer. The effect of substrate concentration on alkaline amylase activity was determined by incubating the reaction mixture for 15 min with different substrate concentration, ranging from 0.5 mg/mL to 4 mg/mL. The effect of enzyme concentration on alkaline amylase activity was determined by incubating the reaction mixture (pH 10) for 15 min at different enzyme concentration ranging from 0.5mL to 4mL. The activity of the amylase was then measured as per assay procedure.

RESULTS AND DISCUSSION

In the present study, a total six cultures were isolated from water and sediment sample of alkaline Lonar Lake, screened for amylolytic activity on starch agar medium and one prominent amylase producer bacterial strain DHT13 was selected on the basis of cultural, morphological, biochemical characteristics and 16s rRNA gene sequencing and partial characterization and optimization of amylase production was performed with this isolate (Fig. 1). In the present studies the phenotypic and 16S rRNA analysis of representative isolates indicated that the bacterial isolates is *Bacillusflexus* (DHT13) (Table 1). The phylogenetic tree based on a comparison of the 16S ribosomal sequences of Lonar Lake isolate DHT13 is shown in table 2. The alkaline amylase producing *Bacilli* are great importance in industries due to its high thermostability and pH stability. The isolated *Bacillus flexus* alkaline amylase production was maximum at pH 10. The effect of enzyme concentration on the activity amylase was observed 2.5mg/mL. As the concentration of enzyme is increases the velocity of the enzymatic reaction also increases but upto certain limit (fig. 2). The optimum substrate concentration of amylase was found to be 0.9mg/mL. The activity of enzyme increases the substrate concentration but this increases up to a certain limit (fig. 3). Production of amylase by *Bacillus flexus*was optimum at 60°C temperatures. At 70°C temperature the production of amylase was low (fig. 4). Optimum pH for the amylase production by *Bacillus flexus*was found to be optimum at pH 10. As pH was decline on either side of pH 10, there was gradual decrease in growth of the organism and production of amylase (Fig.5). The isolated bacteria *Bacillus flexus*produces the amylase enzyme which has thermophilic, alkalophilic and has potential to be used in industry.

Same results reported by Shanmughpriya*et al.,* [1] though their strain was an alkalophilic amylase producer marine isolate *Halobacteriumsalinarum*, it showed the amylase production in the medium supplemented with 2% NaCl. Similar results also reported by Haifeng*et al.,* [8] on the production of amylase from the marine yeast *Aureobasidiumpullulans* N13d. Annamalai*et al.,* [9] reported on amylase production from *Bacillus cereus* and optimum activity was found at pH 8.0 and maintained at pH 11. The *Bacillus* sp. GM8901 was found extremely alkaliphilic a-amylase produced at pH was 11-12 [10, 11] isolate *Bacillus pseudofirmus* and optimum activity was found to be at 80°C, pH 10.0, and 2% NaCl. Tambekar*et al.,* [12] isolates amylase producing *Bacillus sp*. and optimum activity was found to be at 50°C, pH 10.0 and the substrate concentration of amylase 1.45mg/mL.

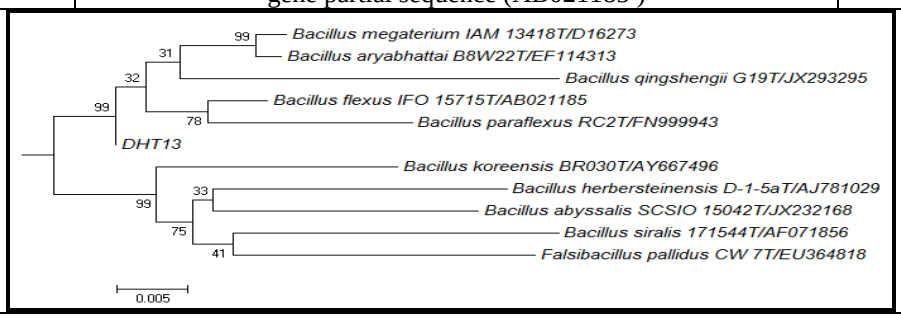
Table 1. Morphological and biochemical characteristics of bacteria isolated from Lonar Lake

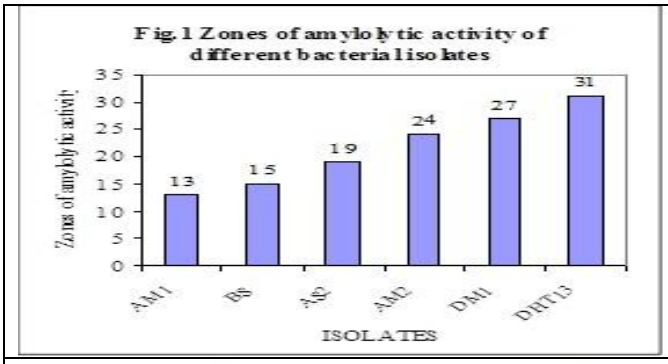
Test	Result	Test	Result	Test	Result
Shape	Rod	Catalase	+	VP	-
Colour of colony	Milky White	Oxidase	+	Arginine	+
Gram staining	+ve	citrate	-	Sucrose	+
Texture	Smooth	Nitrate reduction	+	Maltose	+
Arrangement	Single	Lactose	-	Fructose	+
Motility	Motile	Xylose	+	Dextrose	+
Growth at different temperature		Glucose	+	Melibiose	+
30^0c	+	Arabinose	-	Mannose	+

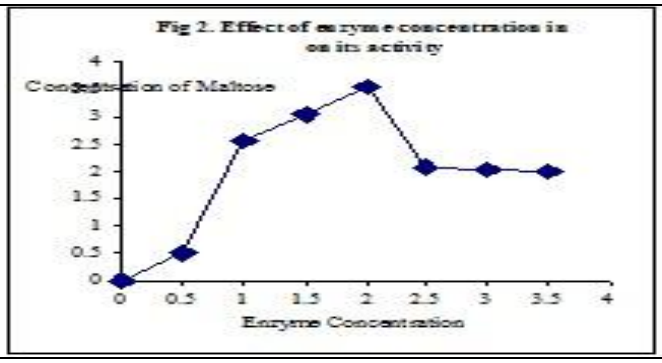
40^0c	+	Saccharose	-	Sodium Gluconate	+
50^0c	+	Galactose	+	Glycerol	-
Growth at different pH		Raffinose	+	Salicin	-
pH 7	+	Trehalose	+	Dulcitol	-
pH 8	+	Mannitol	+	Inocitol	-
pH 9	+	Adonitol	-	Sorbitol	+
pH 10	+	Lysine Utilization	-	Erythritol	-
pH 11	+	Ornithine	-	Melezitose	-
Growth at different salt conc.		Esculin hydrolysis	-	α- Methyl-D-Glucoside	-
1%	+	Rhamnose	-	Xylitol	-
2%	+	Cellibiose	-	Sorbose	-
3%	+	ONPG	-	L-Arabinose	-
4%	+	Esculin	-	Inulin	-
5%	+	Malonate	-	MR	-
Note:- Positive(+) ; Negative(-)					

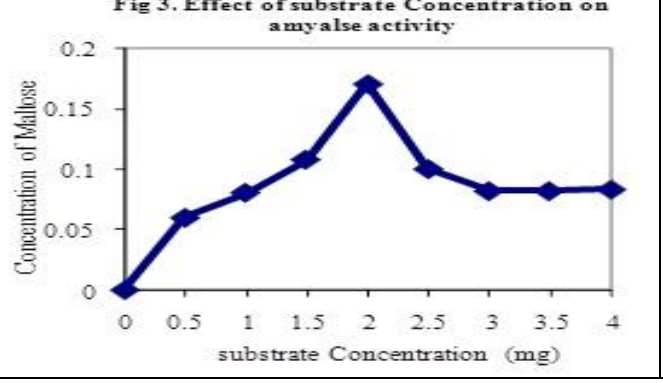
Table 2. The 16S rRNA gene sequencing Closest phylogenetic affiliation and pair similarity of isolated phenol degrading organism from Lonar lake

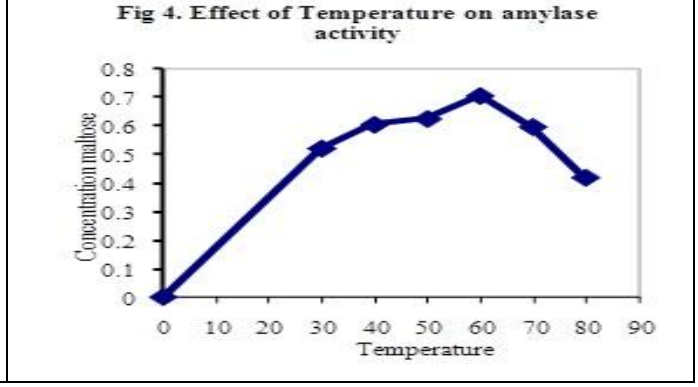
DHT 13	*Bacillus flexus* IFO 15715(T) 16S ribosomal RNA gene partial sequence (AB021185)	99.70%

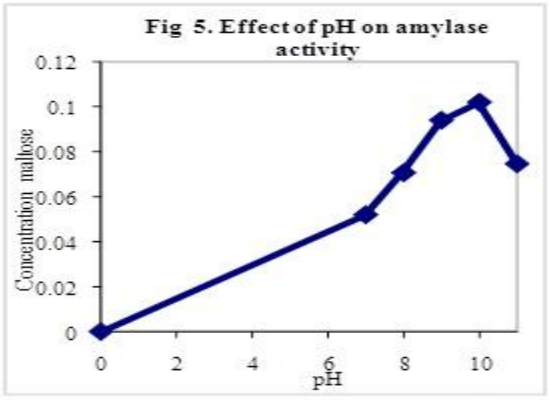












CONCLUSION

Amylase is one of the most important industrial enzymes (approximately 25% of enzyme market) and finds potential applications in food, pharmaceutical and detergent industries. This study reports production of amylase isolated from alkaline Lonar Lake. Amylase from presently isolated *Bacillus flexus*is active at high temperature, 55°C and pH 10 and finds potential applications in food, pharmaceutical and detergent industries.

REFRENCES

1. Shanmughapriya S, Kiran G S, Selvin J, Gandhimathi R, Baskar T B, Manilal A and Sujith S. "Optimization, production and partial characterization of an alkaliphilic amylase produced by sponge associated marine bacterium *HalobacteriumSalinarum.*MMD047. *Biotechnology and Bioprocess Engineering*, 14, 2009, 67-75.
2. Kanekar PP, Nilegaonkar SS, Sarnaik SS and Kelkar AS. Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India. *BioresourceTechnol*, 85(1), 2000, 87- 93.
3. Borsodi AK, Micsinai A, Rusznyak A, Vladar P, Kovacs G, Toth EM, and Marialigeti K. Diversity of alkaliphilic and alkali tolerant bacteria cultivated from decomposing reed rhizomes in Hungarian Soda Lake. *Microbecol*, 50, 2005, 9-18.
4. Tambekar DH and Dhundale VR. Studies on the physiological and cultural diversity of *bacilli* characterized from Lonar Lake (MS) India. *Biosci Disco*, 3(1), 2012, 34-39.
5. Tambekar DH and Tambekar SD. Partial characterization and optimization of alkaline protease production of *Bacillus flexus*from Lonar Lake. *Int J AdvPharm BiolSci*, 2(1), 2012, 130-138.
6. Srinivasan TR, Das S, Bal KV, Philip R and Kannan N. Isolation and characterization of thermostable protease producing bacteria from tannery industry effluent. *Recent Res SciTechnol*, 1(2), 2009, 63-66.
7. Sneath PHA. Endospore forming Gram Positive Rods and Cocci.*Bergey's manual of systematic bacteriology*, 1, 1986, 1104– 1207.
8. Haifeng LI, Zhenming C, Xiaohong W and Chunling MA. Amylase production by the marine Yeast *Aureobasidiumpullulans* N13d. *J Ocean University China*, 6(1), 2007, 60-65.
9. Annamalai N, Thavasi R, Vijayalakshmi S and Balasubramanian T. Extraction, purification and characterization of thermostable, alkaline tolerant a-Amylase from *Bacillus cereus*. *Ind J Microbio*, 51(4), 2011, 424–429.
10. Kim TU, Gu BG, Jeong JY, Byun SM and Shin YC. Purification and characterization of a maltotetraose forming alkaline amylase from an alkalophilic*Bacillus* sp. GM8901. *Appl Environ Microbiol*, 61, 1995, 3105-3112.
11. Dhundale VR, More VR, Nagarkarand RD, Hemke VM and Tambekar DH. Isolation and characterization ofa novel amylase from *Bacillus pseudofirmus. Indian J.L.Sci*, 4 (1), 2014, 69-76.
12. Tambekar DH, Chandurkar AY, Tambekar SD. Partial characterization and optimization of alkaline Amylase from *Bacillus* spp. from Lonar Crater. *Int J Adv Pharm BiolChem*, 3(2), 2014, 481-486.