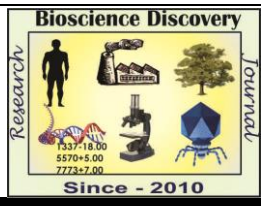
Bioscience Discovery, 8(2):206-210, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article



Extraction and Partial characterization of thermostable, alkaline tolerant a-amylase from *Bacillus oryzaecorticis*

Tambekar DH*, Tambekar SD[1], Jadhav AS and Bhule PB

PG Dept. of Microbiology SGB Amravati University,
Amravati 444602 (M.S.) India
[1]Dept. of Microbiology, Dhote Bandhu Science College, Gondia, 441614
*diliptambekar@rediffmail.com

Article Info

Received: 05-03-2017,
Revised: 27-03-2017,
Accepted: 29-03-2017

Keywords:
Amylase, *Bacillus*, Enzyme, Haloalkaliphilic, Pharmaceutical.

Abstract

Microbial amylase is important enzyme and it constitutes about 60% of total enzyme market. Since, most of the processes in industries are carried out in high alkaline condition and need enzyme with high thermo and pH stability. Therefore attempt was made to isolate alkaliphilic bacillus spp. from Lonar Lake which harbors various unidentified, unique haloalkaliphilic bacterial species which can produce industrially important enzyme. In the present study total 12 samples (sediment, matt and water) were collected from Lonar Lake and enriched on Horikoshi medium (A, B, C and D) and screened for production of amylase on starch agar medium. Out of the isolate, a DHT-24 strain was prominent amylase producer which is identified by 16S rRNA gene sequencing as *Bacillus oryzaecorticis*. The alkaline amylase produced by *Bacillus oryzaecorticis* showed optimum activity at pH 10 and temperature 60^0C. The optimum substrate concentration 2.5 µg/mL and enzyme concentration 2 µg/mL was observed. It proves that the enzyme alkaline amylase produced by this bacillus has potential applications in food, pharmaceutical, textile industries and biotechnological exploitation.

INTRODUCTION

Enzymes are macromolecular biological catalysts which accelerates or catalyze chemical reactions and are very much useful in industries as specialized proteins. It catalyzes more than 5,000 biochemical reaction and every metabolic process in the cell needs enzymes in order to perform at fast enough to sustain life (Stryer *et al.,* 2002, Schomburg *et al.,* 2013). There are various types of enzyme such as amylase; aminoacylase, catalase, cellulase, glucoamylase, glucoisomerase, lactase, lipase, protease, papain, rennet and these enzymes are mostly used in industries. Most of the processes in industries are carried out in high alkaline condition and need enzyme with high thermo and pH stability. Alkaliphilic microorganisms, in particular *Bacillus* species, have much concern in the past decades because of their ability to produce extracellular enzymes that are active and stable at alkaline and thermophilic condition (Horikoshi, 1999). Microbial amylase is important enzyme and it constitutes about 60% of total enzyme market (Kanekar *et al.*, 2000; Borsodi *et al.*, 2005). It have potential application in a number of industrial processes such as pharmaceutical, textile, paper and detergent industries and it has wide application in many other field such as clinical, medical and analytical chemistry (Horikoshi *et al.*, 2006; Tambekar and Dhundale, 2012). Thus alkaline amylase producing bacteria have great significance from industrial point of view. As there is large demand of amylase in industries, isolation and production of amylase enzyme is important to fulfill

this demand (Srinivasan *et al.,* 2009; Tambekar *et al.,* 2015).Therefore attempt was made to isolate alkaliphilic bacillus spp. from Lonar Lake. The alkaline Lonar Lake (Buldhana district, Maharashtra) is saline and alkaline and harbors various unidentified, unique haloalkaliphilic bacterial species. The data showed that these Lonar Lake bacteria can produce industrially important enzyme which are thermostable, resistance to alkali and most the denaturing chemicals. Very less study have been done on amylase from *bacilli* of Lonar Lake which can withstand at high pH as well as high temperature (Tambekar *et al.,* 2014, Tambekar *et al,* 2013,). Therefore aim of the present study is to deal with isolation, production and partial characterization of amylase from bacterial strain isolated from the alkaline Lonar Lake.

MATERIALS AND METHODS

Isolation, screening and identification of isolates: A total of 12 samples of sediment, matt (in zip lock polythene bags) and water (in sterile bottles) were collected from different sites of Lonar Lake. One gram of sediment and matt sample was transferred to 100mL sterilized distilled water in 250 mL conical flask and agitated (100 rpm) at 37^0C for 15 min on rotary shaker. These water suspensions were heated at 80^0C for 10 min to destroy all the vegetative microbial cells and then diluted sample was inoculated in Horikoshi medium (A, B, C and D) for 72h and four time repeated sub culturing was made in the same medium. After enrichment, it was sub-cultured on Nutrient agar plates (pH 10) and incubated at 37^0C for 24h for isolation of pure bacterial colonies. Isolated bacterial colonies were screened for amylotic activity on starch agar medium and the zone of starch hydrolysis was recorded. Then bacterial identifications were analyzed morphologically, culturally, biochemically and 16S rRNA gene sequencing.

Preparation of crude enzyme extract: The 100 mL of starch nutrient broth was inoculated with isolated bacterial culture and incubated at 37°C for 48h. After incubation, centrifuged the broth at 5000 rpm for 15 min. the supernatant served as crude enzyme.

Amylase assay: The standard graphs of maltose was prepared by adding different concentration of maltose and 2 mL of DNS solution into all the test tubes and incubate all the tubes in boiling water bath for 5 min and then addition of 1 mL Na-K tartarate in all tubes to stop reaction. Amylase assay was carried out with 2.5 mL of (1%) starch solution, 2.5 mL of PO_4 buffer, 1 mL of NaCl and 1 mL of enzyme source in a test tube and incubated in boiling water bath for 5 min and then addition of 1mL of Na-K tartarate to stop the reaction.

Characterization of Amylase:

Effect of pH on alkaline amylase activity: The effect of pH on alkaline amylase was determined by assaying the enzyme activity at different pH ranging from 7.0 to 11.

Effect of temperature on alkaline amylase activity: The effect of temperature on alkaline amylase was determined by incubating the reaction mixture at different temperature ranging from 40°C to 80°C for 15 min.

Effect of substrate concentration on alkaline amylase activity: The effect of substrate concentration on alkaline amylase activity was determined by incubating the reaction the reaction mixture at 37°C for 15 min. with different substrate concentration ranging from 0.5 mg/mL to 4.0 mg/mL.

Effect of enzyme concentration on alkaline amylase activity: The effect of enzyme concentration on alkaline amylase activity was determined by the incubating the reaction mixture at 37°C for 15 min with different enzyme concentration, ranging from 0.5 mg/mL to 4.0 mg/mL.

RESULTS AND DISCUSSION

In the present study, a total of 12 different bacterial species were isolated from water, sediment and matt samples from Lonar Lake. Out of 12, six isolates were showed maximum starch hydrolysis activity on starch agar medium at pH 10. Out of them one isolate DHT 24 was selected for further study since it showed prominent amylolytic zone of 23mm (Fig.1). This isolate was characterized culturally, morphologically and biochemically by commercially available Hi-media Rapid detection kit (Table.1) and 16S rRNA gene sequencing and identified as *Bacillus oryzaecorticis* (**KF548480)**. **Joshi *et al.*, (2007)** applied a culture-dependent strategy to explore the diversity of aerobic bacteria of Lonar Lake. Out of one hundred and ninety six bacterial strains, Sixty-four isolates were subjected to phenotypic, biochemical characterization and 16S rRNA sequencing. The phylogenetic analysis indicated that most of the Lonar Lake isolates were related to the phylum Firmicutes, containing Low G+C, Gram-positive bacteria, with different genera.

From the data it showed that, the maximum amylolytic activity was observed at pH 8 (Fig.4) and temperature 60^0C (Fig.3) by *Bacillus oryzaecorticis*. Annamalai *et al*. (2011), reported on amylase production at optimum activity was found at pH 8 and maintained up to pH 11. The optimum enzyme concentration required for optimum activity of amylase was found to be 2 ug/mL (Fig.6) and substrate concentration was found 2.5 µg/mL (Fig.5). Similarly, amylase with optimum activity under alkaline conditions has been studied from the various soda lakes (Martins *et al.*, 2001; Hashim *et al.*, 2004, Tambekar *et al.*, 2013).

Table: 1. Cultural, morphological and biochemical characteristics of Amylase producing *Bacillus oryzaecorticis*

Gram character	+	Glucose	+	α-Methyl-D-glucoside	-
Shape of Bacteria	LR	Dextrose	+	Rhamnose	-
Arrangement of Cell	Single	Galactose	-	Cellobiose	-
Spore	+	Raffinose	-	Melezitose	-
Motility	+	Trehalose	-	α-Methyl-D-mannoside	-
Catalase	-	Melibiose	-	Xylitol	-
Oxidase	-	Sucrose	-	ONPG	-
Voges Proskauer	-	L- Arabinose	-	Esculin hydrolysis	-
Citrate utilization	+	Mannose	-	D-Arabinose	-
Nitrate reduction	-	Inositol	-	Malonate Utilization	+
Arginine	+	Sorbitol	-	Sorbose	-
Lactose	-	Mannitol	-	Inulin	-
Xylose	-	Adonitol	-	Sodium gluconate	-
Maltose	-	Arabitol	-	Glycerol	-
Fructose	-	Erythritol	-	Salicin	-

Note: LR- Long Rod, (+) = Positive, (-) = Negative

Sequence of DHT24

cggagggagatgtcgacttggaggttgtgcccttgaggcgtggcttccggagctaacgcgttaagtcgaccgcctggggagtacggccgcaaggtt
aaaactcaaatgaattgacgggggcccgcacaagcggtggagcatgtggtttaattcgatgcaacgcgaagaaccttacctggtcttgacatccacg
gaagtttccagagatggaaaggtgccttcgggaactgtgagacaggtgctgcatggctgtcgtcagctcgtgttgtgaaatgttgggttaagtcccgc
aacgagcgcaacccttatcctttgttgccagcggtccggccgggaactcaaaggagactgccagtgataaactggaggaaggtggggatgacgtc
aagtcatcatggcccttacgaccagggctacacacgtgctacaatggcgcatacaaagagaagcgacctcgcgagagcaagcggacctcataaag
tgcgtcgtagtccggattggagtctgcaactcgactccatgaagtcggaatcgctagtaatcgtggatcagaatgccacggtgaatacgttcccgggc
cttgtacacaccgcccgtcacaccatgggagtgggttgcaaaagaagtagtagcttaaccttcgggagggcgcttaccactttgtgattcatgactgg
gtgagtctacaggaacccaaaaccgtgtagaaacaaaggtttttcaacatctaaagctgctggtctgcctacaagggaccccaccttccgccggcgtc
ctcccccaaaatctatccattttgcacgtctgccctggaattctagccctctgacaactcatgctgcatacaagagctttcccgtgtagaccagtatttaca
ttctatgaacacgcgggtgctatagcgctaatatcgatacgtagacctatataccggctgggagtatcagcgtcctccggaaaccgtaaaggtaattca
cttccggaagttaaccagtcttcacggttgca

phylum Firmicutes (0/4/5)
class Bacilli (0/2/2)
order Bacillales (0/2/2)
family Bacillaceae 1 (0/1/1)
genus Bacillus (0/1/1)
S004055259 **0.284 1067 Bacillus oryzaecorticis (T); R1; KF548480**

Fig: 1. Plates showing zones of starch hydrolysis

Fig:2. Zone of hydrolysis on starch agar medium

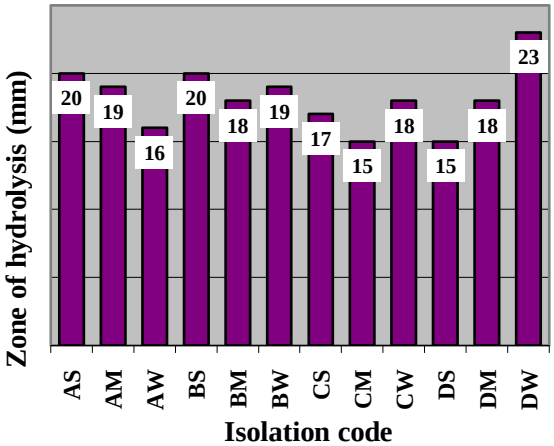


Fig:3. Effect of temperature on the activity of enzyme Amylase

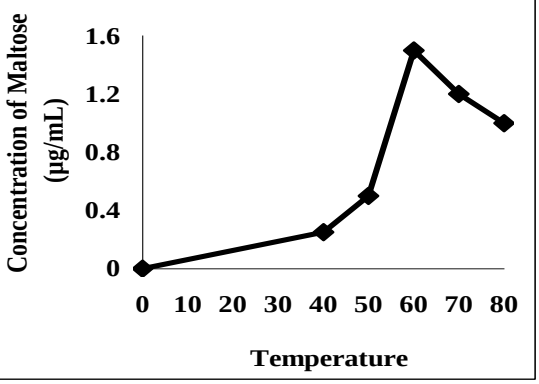


Fig:4. Effect of pH on the activity of enzyme amylase

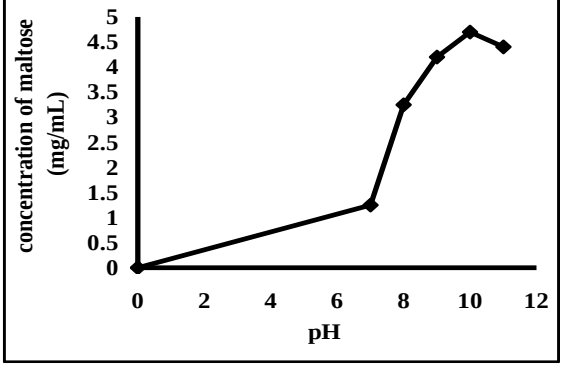


Fig:5. Effect of Substrate on the activity of enzyme Amylase

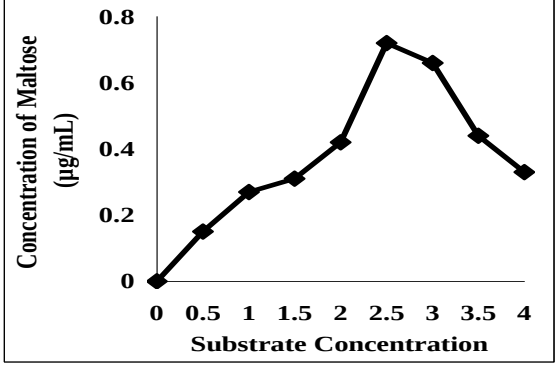
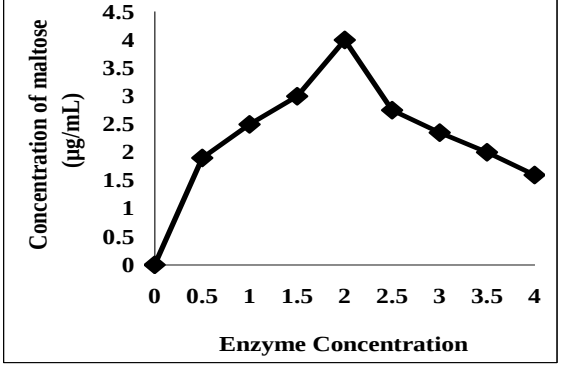


Fig:6. Effect of enzyme concentration on the activity of enzyme Amylase



The microbial diversity of saline lakes has been studied primarily by focusing on the isolation and characterization of individual organisms with potential industrial application. Out of six bacterial strains, one bacterial species was found amylase producer, these alkaline enzyme may be useful in wide industrial and biotechnological interest due to the fact that enzyme are better suited for industrial process. The finding of this study provide a window into the diversity of *Bacilli* community members which were enzyme producing from the Lonar lake. The isolated *Bacillus oryzaecorticis* strain produces the amylase enzyme which was thermostable, alkaliphilic and has potential to produce good quality amylase which can be used in food, pharmaceutical, textile industries and biotechnological exploitation.

REFERENCES:

Annamalai N, Thavasi R, Vijayalakshmi S and Balasubramanian T, 2011. Extraction, purification and characterization of thermostable, alkaline tolerant a-Amylase from *Bacillus cereus*. *Ind J Microbio*, **51**(4): 424–429.

Borsodi AK, Micsinai A, Rusznyak A, Vladar P, Kovacs G, Toth EM and Marialigeti K, 2005. Diversity of alkaliphilic and alkali tolerant bacteria cultivated from decomposing reed rhizomes in Hungarian Soda Lake. *Microb ecol.*, **50**: 9-18.

Hashim SO, Delgado O, Kaul RH, Mula FJ and Mattiasson B, 2004. Starch hydrolyzing *Bacillus halodurans* isolates from a Kenyan soda lake. *Biotechnol.Lett*, **26:** 823–828.

Horikoshi K, 1996. Alkaliphiles–from an industrial point of view. *FEMS Microbiol. Rev*, **18**:259–270.

Horikoshi K, 1999. Alkaliphiles: some applications of their products for biotechnology. *Microbiol Mol Biol Rev*, **63**:735–750.

Horikoshii K, Diurak M, Kojima M, Kanai M and Inoue A, 2006. Isolation and characterization of a feather-degrading enzyme from *Bacillus pseudofirmus*. *Extremophiles*, **10:**229–235.

Joshi AA, Kanekar PP, Kelkar AS, Sarnaik SS, Shouche Y and Wani A, 2007. Moderately halophilic, alkalitolerant *Halomonas campisalis* MCM B-365 from Lonar Lake, India. *J Basic Microbiol*, **47** (3): 213–221.

Kanekar PP, Nilegaonkar SS, Sarnaik SS and Kelkar AS, 2000. Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India. Bioresource Technol, **85(1)**: 87- 93.

Martins RF, Davids W, Abu Al-Soud W, Levander F, Radstrom P and Hatti-Kaul R, 2001. Starch hydrolyzing bacteria from Ethiopian soda lakes. *Extremophiles,* **5:** 135–144.

Schomburg I, Chang A, Placzek S, Sohngen C, Rother M, Lang M, Munaretto C, Ulas S, Stelzer M, Grote A, Scheer M and Schomburg D, 2013. "BRENDA in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA". *Nucleic Acids Research* **41**: 764–72.

Srinivasan TR, Das S, Balakrishnan V, Philip R and Kannan N, 2009. Isolation and characterization of thermostable protease producing bacteria from tannery industry effluent. *Rec Res Sci Technol*, **1**(2): 63-66.

Stryer L, Berg JM and Tymoczko JL, 2002. *Biochemistry* (5th Ed.). San Francisco: W.H. Freeman. 0-7167-4955-6.

Tambekar DH and Dhundale VR, (2012). Studies on the physiological and cultural diversity of *bacilli* characterized from Lonar Lake (MS) India. *Bioscience Discovery*, **3**(1):34-39.

Tambekar DH, Chandurkar AY and Tambekar SD, 2014. Partial characterization and optimization of alkaline Amylase from *Bacillus* spp. from Lonar Crater. *Int J Adv Pharm Bio Chem*, **3**(2): *481-486.*

Tambekar DH, Raut NS and Dhundale VR, 2013. Partial characterization and optimization of alkaline amylase production by *Bacillus circulance* from Lonar Lake. *Bioscience Discovery* **4**(2): 202-207.

Tambekar DH, Tambekar SD and Mohad NA, 2015. Production and dynamics of amylase from *Bacillus flexus. Ind J Pharm Sci*, **5**(3):195-198.

How to Cite this Article:

Tambekar DH, Tambekar SD, Jadhav AS and Bhule PB, 2017. Extraction and Partial characterization of thermostable, alkaline tolerant a-amylase from *Bacillus oryza ecorticis* . *Bioscience Discovery*, **8**(2):206-210