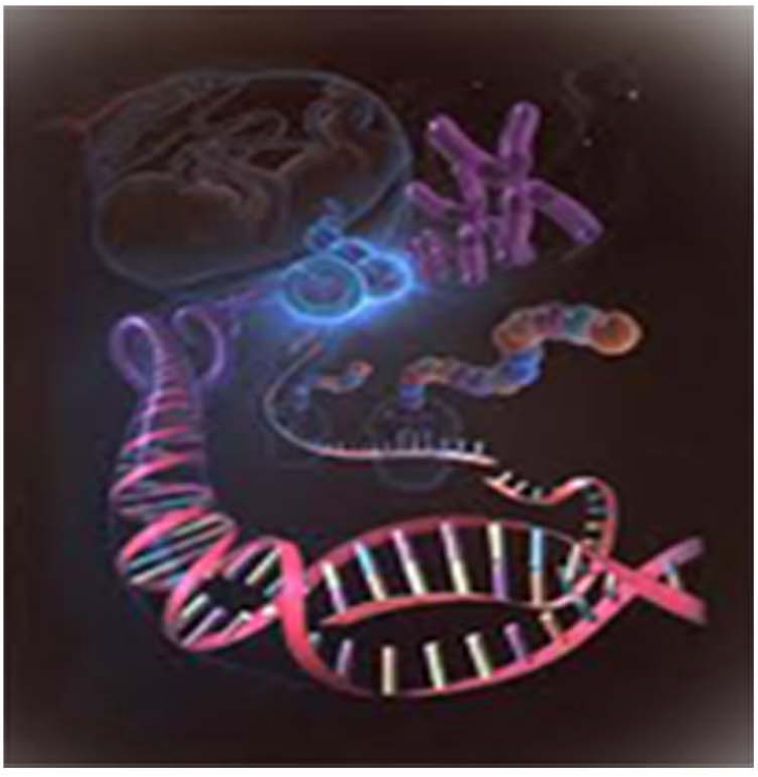
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 3, July 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 3, July 2013

Research Paper

PARTIAL CHARACTERIZATION AND OPTIMIZATION OF LIPASE PRODUCTION FROM *BACILLUS CEREUS* ISOLATED FROM HALOALKALIPHILIC LONAR LAKE

Tambekar D H[1]*, Mundekar S P[1] and Bombode V B[1]

*Corresponding Author: Tambekar D H, ✉ : diliptambekar@rediffmail.com

In India the Lonar Crater Lake, popularly called as the Lonar Soda Lake is situated in the Buldhana District of the Maharashtra State, which is occupied by saline water and harbors various unidentified, unique haloalkaliphilic bacterial *bacillus* species which produces thermo-haloalkaliphilic enzyme. The present study deals with isolation, production and partial characterization of lipase producing bacterial culture isolated from the alkaline Lonar Lake. Total 28 bacterial cultures were isolated by using different enrichment media. Out of 28, 11 bacterial strains were selected on the basis of their lipolytic activity and then studied their phenotypic and biochemical characters. The strain OCW3(1) was selected for 16S rDNA sequencing and production, partial characterizations of lipase on the basis of their maximum lipolytic activity. Phylogenetic analysis based on 16S rDNA gene sequences indicated that strain OCW3(1) was affiliated to phylum Firmicutes with genera *Bacillus.* Newly isolated *Bacillus cereus* OCW3(1) strain was found to optimum production lipase at pH 9 and optimum temperature was found at 60°C. The best substrate concentration 2.33 unit/mL and enzyme concentration 4.66 unit/mL were observed. The abilities of *Bacillus cereus* OCW3 (1) to produce extracellular lipase suggest that they might be of potential valuable for biotechnological exploitation.

Keywords: Lonar crater, Haloalkaliphiles, Bacillus, Lipase

INTRODUCTION

Alkaline Lonar Lake in India is a unique ecosystem and wonder on the earth being formed by meteorite impact on basaltic rock (Tambekar *et al*, 2010). It is situated in a village Lonar in the Buldhana district of Maharashtra state, India. It has been well-known as an inland saline lake with high concentration of sodium carbonate and chloride (Kanekar *et al.,* 2000). These multi-faceted enzymes have tremendous potential in area such as detergent, food technology, pharmaceutical, leather, chemical industry, biomedical science, cosmetic, industrial waste management and in pulp and paper industry to improve quality, dairy, textile, wastewater treatment (Tambekar and Tambekar, 2011). The

[1] PG Dept. of Microbiology SGB Amravati University, Amravati.

detergent industry is the largest market for this enzyme. It is also thermostable organism growing in naturally alkaline habitats may have lipase with special characteristics. However, attempt was made to isolate new species of bacillus, which can produce good quality of lipase useful in the detergent and leather industry (Horikoshi, 1999).

Alkaline lipase produced by bacteria are of great importance in detergent and leather industry due to their high thermo-stability and pH stability and it has most important industrial enzymes accounting for about 60% of total enzyme market, very low work or study had been perform on lipase producing bacteria, which having abilities on high temperatures with high pH stability and has tremendous useful application in various types of industries. So, it's lead to, there is large demand of Lipase. The present study aims to deal with the isolation, screening, partial characterization and production of a lipase producing bacteria isolated from the alkaline Lonar Lake.

MATERIALS AND METHODS

Collection of Lonar Lake Water Sediment and Matt Sample

Total 12 samples is collected from four different sites, from each site 3 sample were collected water, sediment and matt sample. Water sample collected in sterilized plastic bottle and sediments sample in sterilized plastic bags, sample was transported to laboratory for isolation and identification of bacteria.

Enrichment and Isolation of Microorganisms

Enrichment of water and sediment samples was carried out in Horikoshi I (A), Horikoshi II (B), Peptone 5.0, yeast extract 1.5, beef extract 1.5, sodium chloride 5.0, agar 20.0, pH 10 (C). Peptone 5.0, yeast extract 1.5, beef extract 1.5, sodium chloride 35.0, agar 20.0 pH 10 (D); pH of the medium was adjusted using 1 N NaOH solution. One gram sediment sample from each site was dissolved in sterile distilled water and poured in enrichment medium. All the flasks were incubated at room temperature on a rotary shaker (100 rpm) for 7 days. After enrichment, the organisms were isolated on respective medium agar plates and incubated at 37° C. Well isolated and differentiated colonies from these enrichment media were transferred on the respective media slants and the same were maintained as stocks for further study (Horikoshi, 1999; Joshi *et al.,* 2007).

Screening of Bacterial Alkaliphiles

A specific individual bacterial colonies were screened for lipolytic activities on egg yolk agar plate (egg yolk 1%, Peptone 1%, yeast extract 1%, beef extract 1%, sodium chloride 0.5%, Agar-Agar 2%, pH 10). The pH of the medium was adjusted by using pH meter with addition of 1 N NaOH before and after sterilization (Joshi *et al.*, 2007). The inoculated plates were incubated at 37^0C for 72 h. The halozone uppers near the colony it means that the lipid was hydrolyzed by bacteria.

Identification of Lipolytic Isolates

The bacterial with prominent zone of clearance on egg yolk agar medium were processed for identifications based on morphological, cultural and biochemical characteristics. The isolates were also tested for their growth at different temperature, pH and NaCl concentration. These isolates were identified in accordance with the methods recommended in Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986). The selected strains were then analyzed by 16S rRNA sequencing at NCCS, Pune (Maharashtra) and BLAST Identification was made.

Preparation of Crude Enzyme Extracts

Take the 100 mL of sterile alkaline nutrient broth and 1 mL egg yolk was added. The broth was inoculated with cultures and incubated for 72 h incubation, centrifuged the broth at 5000 rpm per 15 min. The supernatant served as crude enzyme sources for extracellular lipase.

Optimization of Crude Enzyme Lipase

Estimation of lipase was carried out by standard titrimetric method. Assay mixture contain 5 mL oil emulsion and 5 mL 0.1 M tris buffer and added 1 mL enzyme suspension and incubated for 30 min at room temperature. After incubation to stop the reaction by addition of acetone and methanol mixture and titration was done against 0.025 N NaOH by addition of 1% phenolphthalein indicator.

Determination of Lipolytic Activity

Lipase activity in the synthetic media was determined titrimatrically on the basis of olive oil hydrolysis by the slightly modified method of Kempka *et al.* (2008).

Assay mixture containing 180 mL of distilled water, 20 mL olive oil, 0.4 g sodium benzoate with 1 g gum Arabic such assay mixture mix with 5 mL 0.1 M tris buffer (pH 8) and added 1 mL culture supernatant, such type of master mixture incubated at 40^0C for 30 min and the reaction stop with 10 mL of acetone and methanol mixture (1:1). Liberated fatty acids were titrated with 0.025 N NaOH using 1%. Phenophthalein as indicator. The one unit of Lipolytic activity was defined as the amount of enzyme that produced fatty acid in (μmL^{-1}) unit per ml under the standard assay conditions (htttp://www.sigmaaldrich.com).

Lipase Unit Calculation

$$\text{Lipase Activity} = \frac{\Delta V \times N}{V_{sample}} \times \frac{1000}{30}$$

where $\Delta V = V_2 - V_1$

V_1 = Volume of NaOH used against control flask

V_2 = Volume of NaOH used against experimental Flask.

N = Normality of NaOH.

Effect of pH on Alkaline Lipase Activity

The effect of pH on alkaline lipase from *Bacillus* spp. was determined by assaying the enzyme activity at different pH values ranging from 7.0 to 10.5.

Effect of Temperature on Alkaline Lipase Activity

The effect of temperature on the Lipase stability was determined by measuring the residual activity after 30 min of incubation in 0.1 M tris buffer (pH 8) at different temperature ranging from 40^0C to 100^0C.

Effect of Substrate on Alkaline Lipase Activity

The effect of substrate concentration on alkaline lipase activity was determined by incubating the reaction mixture (pH 10.5) for 30 min with different substrate concentration, ranging from 0.5 mL to 8.0 mL.

Effect of Enzyme on Alkaline Lipase Activity

The effect of enzyme concentration on alkaline lipase activity was determined by incubating the reaction mixture (pH 0.5) for 30 min at various enzymes concentration ranging from 1 mL to 8 mL. The activity of the lipase was then measured as per assay procedure.

RESULTS AND DISCUSSION

In the present study a total 28 different bacterial species were isolated from water, sediment and

matt sample of Lonar Lake. Out of 28, 21 bacterial strains were found lipase producer. The strains OCW3(1) were screened for production and partial characterizations of lipase on the basis of their optimum lipid hydrolysis and was found tobe 28 mm (Figure 1). The bacterial strain OCW3(1) was identified according to morphological, cultural and standard biochemical test. In present study *B. cereus* cells revealed in chain arrangement, long rod shape, 5.0 µm in length and 1.0 µm in width, Gram-positive. Cells were highly motile and produce spores which were cylindrically and located at central in unswollen sporangium with capsulated. Colonies of OCW3(1) were characteristically very large 6 mm in diameter and white pigmented, irregular and umbonate elevation with undulate edges were observed. The bacterial strain OCW3(1), growth was detected with different pH 7 to 12 and NaCl concentration ranging from 0-7% NaCl. The growth of strain OCW3(1) was found at 40^0C, 50^0C temperature but on 55^0C the growth was not observed. The bacterial strain shown catalase positive and oxidase negative. Indole and Methyl red showed negative and voges proskauer test were positive and citrate utilization by Simmion's citrate, nitrate was reduced to nitrite and unable to hydrolyzed the urea. Glucose, arabinose, mannitol, trehalose, sucrose, fructose were utilized while, mannitol, xylose, lactose, cellobiose, galactose, maltose, salicin, sorbitol were does not found acid fermentation by OCW3(1) (Table 1). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain OCW3(1) was affiliated with the genus *Bacillus* (Figure 2). The highest similarity values with the sequences of obligately alkaliphilic microorganisms. The phylogenetic analysis based on 16S rRNA gene sequences indicated that the taxonomic position of strain OCW3(1) belonged to the *Bacillus cereus* HMT6 HQ156459. Similar results the *B. cereus* was also reported from the Kenyan, Hungarian Soda Lake (Vergas *et al.,* 2004; Borsodi *et al.,* 2003).

Alkaline lipase production was maximum at pH 9-10.5. Maximum lipase production was recorded after 48 h of incubation at 37°C. The optimum pH for maximum activity of lipase was found to be 9 (Figure 3). The strain B. cereus OCW3(1) produced thermostable lipase. The enzyme was thermostable at 60°C and retained its optimum activity after incubation of 30 min at 60°C (Figure 4). The optimum enzyme concentration required for maximum activity of lipase was 4.66 unit/ml (figure 5). In the effect of substrate concentration on enzyme activity of lipase was found to be 2.33 unit / mL (Figure 6). Both alkali-tolerant and obligate alkaliphiles were found and identified by phylogenetic analysis as the microbial species found in soda lake microbial population and known for being good lipase producers (Tambekar and Dhundale, 2012; Vargas *et al.,* 2004). Such thermal stability distinguishes the lipase produced by strain *B. cereus* OCW3(1) from other thermostable lipases producing bacilli (Ghanem *et al*., 2000). Microorganisms that survive in extreme condition especially at elevated pH (10-12) are able to produce alkaline enzymes that in general show high catalytic activity at the optimal growth conditions (Tambekar *et al.,* 2012). Few moderate alkaliphilic strains especially representatives of the genus *Bacillus*, are able to produce extracellular amylase, lipase and proteases that are even active at alkaline pH (Martins *et al.,* 2001; Vargas *et al*., 2004; Tambekar and Tambekar, 2012). A large number of alkaliphilic *Bacillu*s strains have been isolated for industrial applications (Horikoshi, 1971).

Table 1: Biochemical Characteristic of *Bacillus cereus* OCW3(1) Isolated from Lonar Lake

Morphology	Gram nature	+		pH 7	+	Utilization	Glucose	+
	Shape of cell	Rod		pH 8	+		Arabinose	+
	Arrangement	Chain	Growth at pH	pH 9	+		Mannitol	–
	Motility	+		pH 10	+		Xylose	–
	Capsule	+		pH 12	+		Lactose	–
Spore	Spore	+		45ºC	+		Sucrose	+
	Position	Central		50ºC	+		Cellobiose	–
	Shape	Oval	Growth at Temperature	55ºC	–		Galactose	–
	Swollen Sporangium	–		Catalase	+		Maltose	–
Cultural characters	Pigment	White		Oxidase	–	Hydrolysis of	Fructose	+
	Colony Shape	Irregular		Indol	–		Salicin	–
	Elevation	Umbonate		MR	–		Sorbitol	–
	Edge	Undulate	Biochemical characters	VP	+		Starch	+
	Internal	Wavy						
	structure	Interlaced		Citrate utilization	+		Lipid	+
Growth at NaCl	2%NaCl	+		Urease	–		Casein	+
	7%NaCl	+		Nitrate reduction	+		DNA	+

Figure 1: Lipolytic Activity of Bacterial Culture Isolated from Lonar Crater

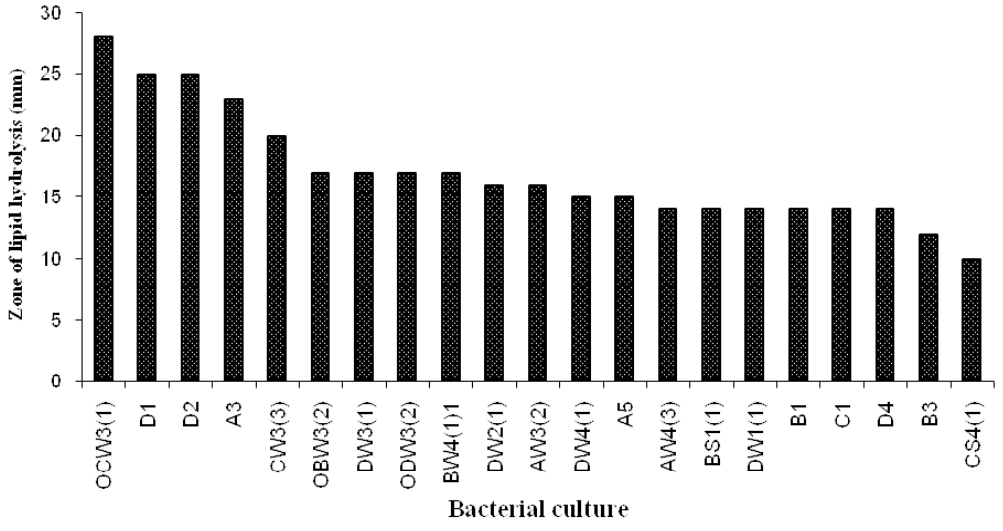


Figure 2: Phylogenetic Tree Based on a Comparison of the 16s Ribosomal DNA Sequences of Lonar Lake Isolates *Bacillus cereus* Ocw3(1) and Some of their Closest Phylogenetic Relatives. The Tree Was Created By The Neighbor-joining Method. The Numbers on The Tree Indicates. The Percentages Of Bootstrap Sampling

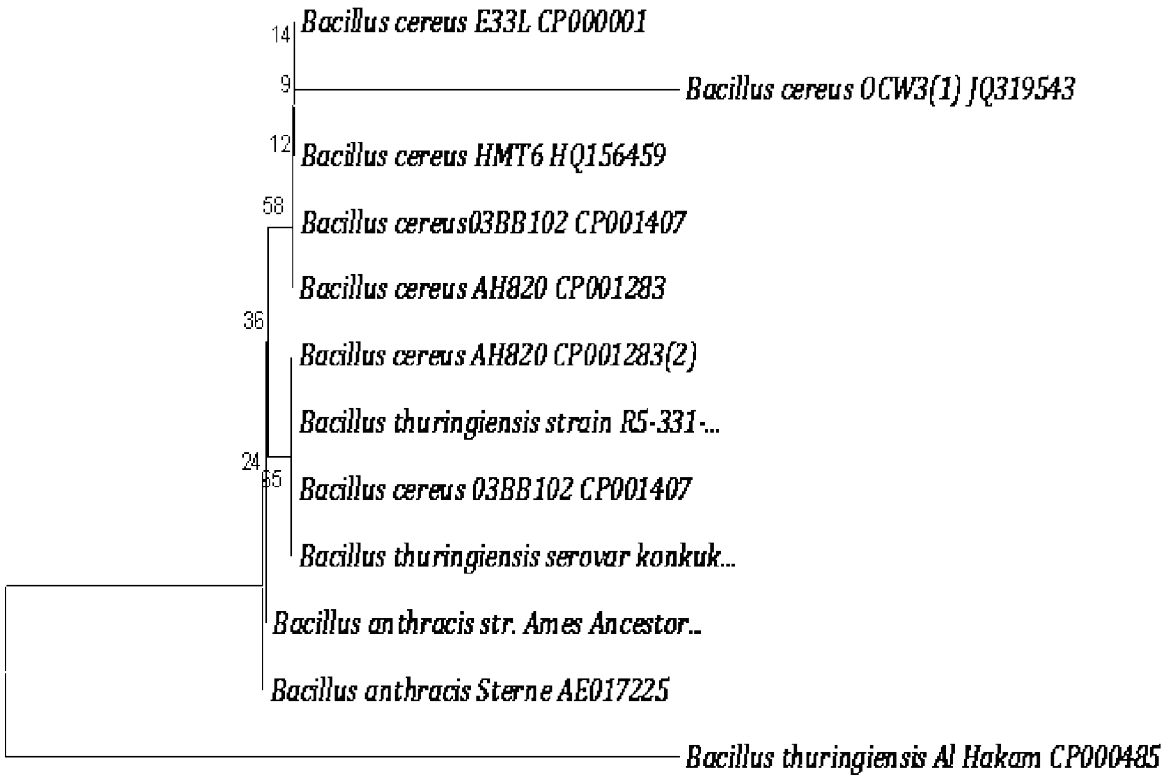


Figure 3: Effect of pH on Lipase

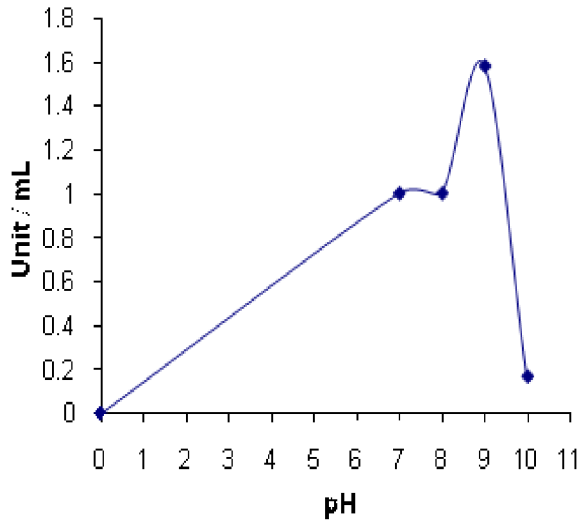


Figure 4: Effect of Temperature on Lipase

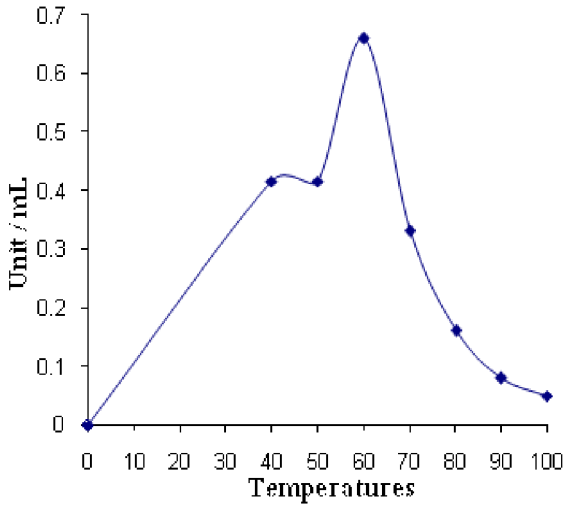


Figure 5: Effect of Enzyme Concentration on Lipase

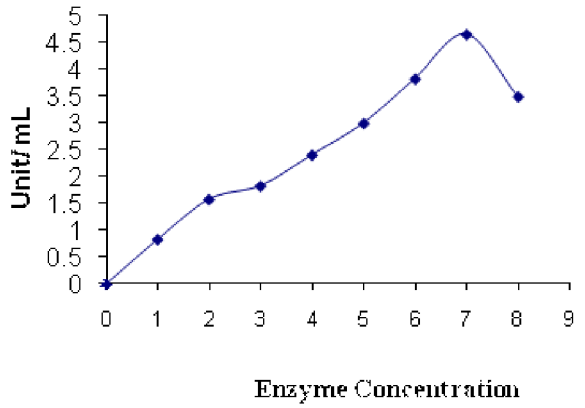
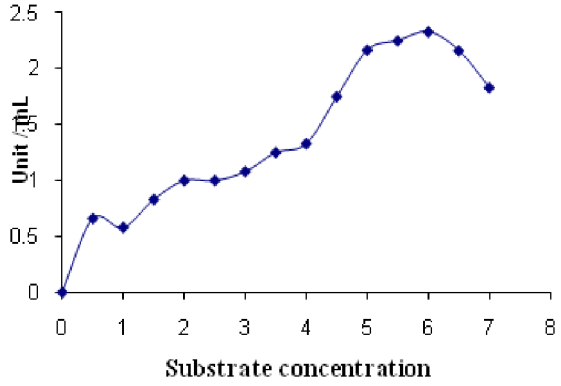


Figure 6: Effect of Substrate Concentration on Lipase



CONCLUSION

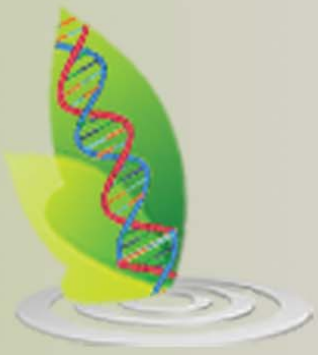
The characteristic point of strain OCW3(1) was production of alkaline thermostable lipase that is optimally active at high pH 9 and thermostable at 60°C. Those characteristics of lipase produced by strain OCW3(1) distinguish from other alkaline and thermostable lipases produced by other bacilli. The isolated *Bacillus cereus* strain produces the lipases enzymes which was thermostable, alkaliphilic and has potential to produce good quality lipases which can be use in the industrial level. The developing novel techniques in genetic engineering combined with better knowledge of structure and function allow fulfillment of industrial needs and exploration of novel applications, genetic characterization of strain *B. cereus* OCW3(1) will encourage future application of genetic methods toward strain development for improved the productivity.

REFERENCES

1. Borsodi A K, Micsinai A, Rusznyak A, Vladar P, Kovacs G, Toth E M and Marialigeti K (2005), "Diversity of Alkaliphilic and Alkali Tolerant Bacteria Cultivated From Decomposing Reed Rhizomes in Hungarian Soda Lake", *Microb Ecol*, Vol. 50, pp. 9-18.
2. Ghanem E H, Hashim AAl-Sayed and Saleh K M (2000), "An Alkalophilic Thermostable Lipase Produced By A New Isolate of *Bacillus alcalophilus*", World *J Microbiol Biotechnol*, Vol. 16, pp. 459-464.
3. Horikoshi K (1999), "Alkaliphiles: Some Applications Of Their Products For Biotechnology", *Microbiol Mol Biol Rev.,* Vol. 63, pp. 735-750
4. Horikoshi K (1971), "Production of Alkaline Enzymes By Alkaliphilic Microorganisms. Part II. Alkaline Amylase Produced by Bacillus no. A-40-2", *Agricultural and Biological Chemistry,* Vol. 35, pp. 1783-1791.
5. Joshi A A, Kanerkar P P, Sarnaik S and Kelka A , Banmeru P K, Banmaru S K and Mishra V R (2005), *Biodiversity of Lonar Crater,* pp. 71-75, Anamaya Publishers, New Delhi, India.
6. Kanekar P P, Nilegaonkar S S, Sarnaik S S and Kelkar A S (2000), "Optimization of Protease Activity of Alkaliphilic Bacteria Isolated From an Alkaline Lake in India", *Bio Resource Technol*, Vol. 85, No. 1,

pp. 87-93.

7. Kempka A P, Lipke N L, Pinheiro T L F, Menoncin S, Treichel H, Freire D M G, Luccio M D and Oliveira D (2008), "Response Surface Method to Optimize the Production and Characterization Of Lipase From *Penicillium Verrucosum* In Solidstate Fermentation", *Biop. Biosys. Eng*., Vol. 31, pp. 119-125.
8. Martins R F, Davids W, Abu Al-Soud W, Levander F, Radstrom P and Hatti-Kaul R (2001), "Starch Hydrolyzing Bacteria From Ethiopian Soda Lakes", *Extremophiles*, Vol. 5, pp. 135-144.
9. Sneath P H A (1986), "Endospore-forming Gram-Positive Rods and Cocci", In: Murray R G E, Brenner D J, Bryant M P *et al.* (Eds.), *Bergey's Manual of Systematic Bacteriology*, 1st Edition, pp. 1104-1207, Williams and Wilkins, Baltimore, Md.
10. Tambekar D H and Dhundale V R (2012), "Studies on the Physiological and Cultural Diversity of *bacilli* Characterized from Lonar Lake (MS) India", *Biosci Disco*; Vol. 3, No. 1, pp. 34-39.
11. Tambekar D H and Tambekar S D (2012), "Partial Characterization and Optimization of Alkaline Protease Production of *Bacillus pseudofirmus* from Lonar Lake", *Int J Adv Pharm Biol Sci*, Vol. 2, No. 1, pp. 130-138.
12. Tambekar D H and Tambekar S D, (2011), "Partial Characterization And Optimization of Protease Production From Newly Isolated *Cohnella thermotolerans* from Lonar Lake", *J res Biol*, Vol. 4, pp. 292-298.
13. Tambekar D H, Pawar A L and Dudhane M N (2010), "Lonar Lake water: Past and Present", *Nat Env Poll Tech*, Vol. 9, No. 2, pp. 17-221.
14. Vargas V A, Delgado O D, Hatti-Kaul R and Mattiasson B (2004), "Lipase Producing Microorganisms From a Kenyan Alkaline Soda Lake", *Biotechnol Lett,* Vol. 26, pp. 81-86.



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137