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Research Article

SCREENING OF ANTIMICROBIAL POTENTIALS OF HALOALKALIPHILIC BACTERIA ISOLATED FROM LONAR LAKE

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ABSTRACT

The Lonar Crater Lake, situated in the Buldhana District of the Maharashtra State of India, is highly saline, alkaline and harbors various unidentified, unique haloalkaliphilic bacterial species which can be produces biotechnological important secondary metabolites. The present study deals with isolation, production and partial characterization of antibacterial substance producing bacteria from the alkaline Lonar Lake. Total twenty nine bacilli were isolated by using different enrichment media, out of which BW1(1) selected for antibacterial study and subjected to phenotypic and biochemical characteristics and identified as *Oceanobacillus iheyensis* on the basis of 16S rDNA sequencing. The strain was found to be potential antibacterial against *E. coli* and moderate against *K. pneumoniae, S. typhi, S. aureus* and poor against *P. aeruginosa, P. vulgaris* and *E. aerogenes.* The study reveals that the haloalkaliphilic *O. iheyensis* produce broad spectrum of antimicrobial agents which can be exploited for biotechnological potential and improve as promising sources for new antibacterial compound.

Keywords: Lonar Lake, *Oceanobacillus iheyensis,* Antibacterial activity.

INTRODUCTION

The Lonar Crater Lake is a Lonar Soda Lake situated in the Buldhana District of the Maharashtra State of India. The uniqueness of Lonar Lake is its salinity and alkalinity which is harbors various unidentified, unique haloalkaliphilic bacterial species which can be produces secondary metabolites[1]. Bacteriocin are antimicrobial peptides widespread produced among bacteria[2]. Despite the intensive work on bacteriocin produced by lactic acid bacteria, the genus *Bacillus* comprises a variety of industrially important species and has a history of safe use in both food and pharmaceutical industry[3]. Alkaliphilic microorganisms, in particular *Bacillus* species, have attracted much interest because of their ability to produce extracellular metabolites that are active and stable at high pH. The unusual properties of these metabolites offer a potential opportunity for their utilization in processes demanding such extreme conditions[4].

The development of resistance to multiple drugs is major issue in the medication of infectious disease caused by pathogenic microorganism. The multidrug resistance is presently targeted on exploration and therefore need to search new bioactive compound. To achieve this conditions and circumstances, there is concern to ameliorate or detect novel class antibiotics that have distinct mechanisms of activity globally[5]. The suitability of secondary microbial products produced from realizable bacterial taxa was significant to discover novel chemicals for the improvement of new therapeutic agents[6, 7]. Halophilic bacteria from marine environment are also better sources of secondary metabolites that may have potential of pharmaceutical and biotechnological applications[8]. The haloalkaliphiles are an exciting subset of extremophilic organisms and represent extremophiles that are adapted to two extreme conditions such as saline and alkaline growth. Tambekar *et al*.[9] studied the antibacterial activity of medicinal plant of Lonar Lake. In Lonar, no significant studies have been conducted so far to isolate and produce useful antibiotics. Therefore, present study is intended to isolate, screen and characterize antibiotic producing bacteria from Lonar Lake which could be further explored for a biotechnological potential.

MATERIALS AND METHODS

Isolation and identification of microorganisms

Thirty two water samples and sixteen sediment samples were collected from Lonar Lake in the sterile sampling bottles. Enrichment strategy was established by four different liquid media. 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) and Peptone 5.0, Yeast Extract 1.5, Beef extract 1.5, Sodium Chloride 35.0, Agar 20.0, pH 10 (D). The water and sediments samples were inoculated 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 A, B, C and D media agar plates and incubated at 37°C. Well isolated and differentiated colonies from these enrichment media were transferred on the respective medium slants and same were maintained as stocks for further study [10; 11]. Bacterial cultures were examined for their morphological, cultural and standard biochemical test according to Bergey's Manual of systematic bacteriology.

16S rDNA Sequencing and Phylogenetic Analysis

The 16S rDNA sequences analysis was carried out at the National Centre for Cell Science, Pune, and sequences were submitted to NCBI GenBank Database for the accession numbers. The phylogenetic tree was constructed from evolutionary distances using the neighbor-joining method of Mega 4 program package[12]. The 16S rDNA sequences were submitted to NCBI GenBank Database. The accession numbers as JQ319526

Cell free culture for Antibacterial activity

Bacterial culture grown in respective broth at 37°C for 48h. Cell suspensions were centrifuged at 5000 rpm for 15min. The antagonistic activity of antibacterial substance was determined by disc diffusion method[13].

Thermal and pH stability of antibacterial substance

Aliquots of cell free supernatant were exposed to temperatures ranging from 60°C, 70°C, 80°C, 90°C, 100°C and 121°C for 10 min. Similarly sensitivity of antibacterial substance to different pH was tested by adjusting the pH of cell free culture in the range of pH 7.0 to 12 then antibacterial activities were detected by disc diffusion method against test organism.

Antibacterial activity using disc diffusion method

Sterile blotting paper discs were dipped into 48h incubated cell free culture broth and then placed on solidified Nutrient agar seeded with 3h old culture of test organism, which includes *Escherichia coli* (MTCC 443), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae* (MTCC 2653), *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 734), *Pseudomonas aeruginosa* (MTCC 424) and *Staphylococcus aureus* (MTCC 96) plates were kept for incubation at 37°C for 24h. Zones of inhibition were measured.

RESULTS AND DISCUSSION

In the present study a total of twenty nine cultures were isolated from water and sediment of Lonar Lake and maintained on respective medium slant (pH 10). The morphological and cultural tests including pH and NaCl (salt) tolerance of isolated bacterial species were studied. All these bacterial strains were Gram positive, spore forming bacilli and found stable upto 45°C and seven were found thermotolerent upto 50°C. All of the alkaliphiles were identified as different species of the genus *Bacillus*, which are known to produce a wide variety of metabolites with tolerance to thermal and alkaline conditions[4].

All the bacterial strains were found both alkaliphilic (7-12) and halophilic (0.5-7% NaCl) and the optimum growth pH was 10 for all the bacterial strains. Then these cultures detected for antagonistic activity against standard pathogenic bacteria. The BW1(1) selected for further characterization and screening for antagonistic activity (Table 1). Bootstrap analysis was used to evaluate phylogenetic tree stability according to a consensus tree from the neighbor-joining based on 1,000 replicates for each. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BW1(1) was affiliated to phylum Firmicutes with genera *Oceanobacillus* (Fig 1). Lonar lake strain BW1(1) was showed high similarity to *O. iheyensis*.

In present study, total twenty nine bacilli strains were analyzed for its antagonistic activity. The twenty six *Bacillus* shown antagonistic activity against *E. coli* (89%), twelve (41%) *Bacillus* species showed antagonistic activity against *K. pneumoniae*, seven (24%) were found antagonistic against *S. typhi*, four (13%) *Bacillus* species were shown antagonistic activity against *S. aureus*. The growth of *P. aeruginosa, P. vulgaris* and *E. aerogenes* were not inhibited by any bacterial strains. Out of twenty nine, BW1(1) select the bacterial strains for further

characterization, screening was done on the basis of pH and salt tolerance maximum antagonistic activity. The cell free culture of *O. iheyensis* were used as crude antibacterial substance and tested against pathogenic bacteria. The prominent antibacterial activity was found against *E. coli* and moderate against *K. pneumoniae*, *S. typhi*, *S. aureus* and poor antibacterial against *P. aeruginosa*, *P. vulgaris* and *E. aerogenes* (Table 2). In present study, antibacterial activity *Bacillus* were mostly inhibitory to Gram-negative strains and less effective against Gram positive bacteria. Lei Chen *et al*. [14] isolate halophilic bacterial strain which had inhibited *B. subtilis* and few halophilic bacterial strain displayed antifungal activity against human pathogenic fungus, *Candida albicans*. During the past two decades, marine bacteria have highlighted the tremendous potential of the microorganisms as a source of new bioactive secondary metabolites [15].

Table 1: Biochemical characteristic of *Oceanobacillus iheyensis* BW1(1) isolated from Lonar Lake

Morphology	Gram character	+	Biochemical characters	Catalase	+
	Shape of Bacteria	Rod		Oxidase	+
	Length	3 µm		Indol	-
	Width	0.6 µm		MR	-
	Arrangement of cell	Chain		VP	-
Spore	Spore bearing	+		Citrate	-
	Position	Central		Urease	-
	Shape	Oval		Nitrate	-
	Swollen Sporangia	-	Utilization of	Glucose	+
	Capsule	+		Arabinose	-
	Motility	Motile		Mannitol	-
Cultural characters	Enrichment medium	Horikoshi II		Xylose	-
	Size	1 mm		Lactose	-
	Pigment	White		Trehalose	-
	Shape	Circular		Sucrose	-
	Elevation	Convex		Cellobiose	-
	Edge	Entire		Galactose	-
	Internal structure	Transparent		Maltose	-
	Colony on slant	Eschinulate		Fructose	-
Growth at pH	pH 7	+		Salicin	-
	pH 12	+		Sorbitol	-
Growth at NaCl	1% NaCl	+		Raffinose	-
	7%NaCl	+	Hydrolysis of	Starch	-
Growth at temperature	50° C	+		Lipid	-
	55° C	-		Casein	+

The heat sensitivity of the antimicrobial substance was determined by measuring its activity after incubation for 10 min at different temperatures. The cell free culture of *O. iheyensis* were exposed at various temperatures it was found stable at 60°C and active against the *E. coli* and *K. pneumoniae* and not shown the antibacterial activity against *S. aureus*, *S. typhi*, *E. aerogenes* and *P. vulgaris* and *P. aeruginosa*. The *O. iheyensis* was found to be antibacterial against *E. coli* and *K. pneumoniae* and stable after heat treatment at 70°C and 80°C and showed the antibacterial activity (Fig 2). Naclerio *et al.* [16] isolated Cerein from *B. cereus* which was partially stable to heat treatment. They revealed that, the activity was maintained during treatment up to 75°C and inactivited only after 15 min incubation at 90°C. In the present study, the cell free culture of *O. iheyensis* was found heat sensitive at 90-121°C and did not shown antibacterial activity against *E. coli* while antibacterial substance were showed antibacterial activity against *K. pneumoniae* at 90-121°C. Our study provides evidence that *Oceanobacillus* strains were promising sources for the bioactive substances and stable at high temperature. These bacteria in general represent a new and rich source of secondary metabolites that need to be explored. In the present study, the antibacterial substance showed activity over a wide range of pH from 7 - 12. The cell free culture of *O. iheyensis* was exposed at various pH and performed antibacterial activity.

At alkaline pH 10-12 the antibacterial activity were found prominent against *E. coli* and moderate against *K. pneumoniae*, *S. typhi*, *S. aureus* and poor against *P. aeruginosa*, *P. vulgaris* and *E. aerogenes*. As pH decreases, an antibacterial activity also decreases or looses (Fig 3). At pH 7-9, the antibacterial substance showed only prominent activity against *E. coli* and moderate activity were found against *K. pneumoniae* while no antibacterial activity were

found against *P. aeruginosa, P. vulgaris, E. aerogenes, S. typhi* and *S. aureus.* Mean while antibacterial substances which were isolated strains needed alkaline pH for antibacterial activity. Similar study also done by Khalil *et al.* [17] on the inhibitory compound produced by *B. megaterium* was also stable at pH 2-7, but the activity was totally lost at alkaline pH value of 8 and above. This result was in consistence with those reported by Todorov *et al.* [18] on *L. lactis* subsp. *lactis* bacHV219.

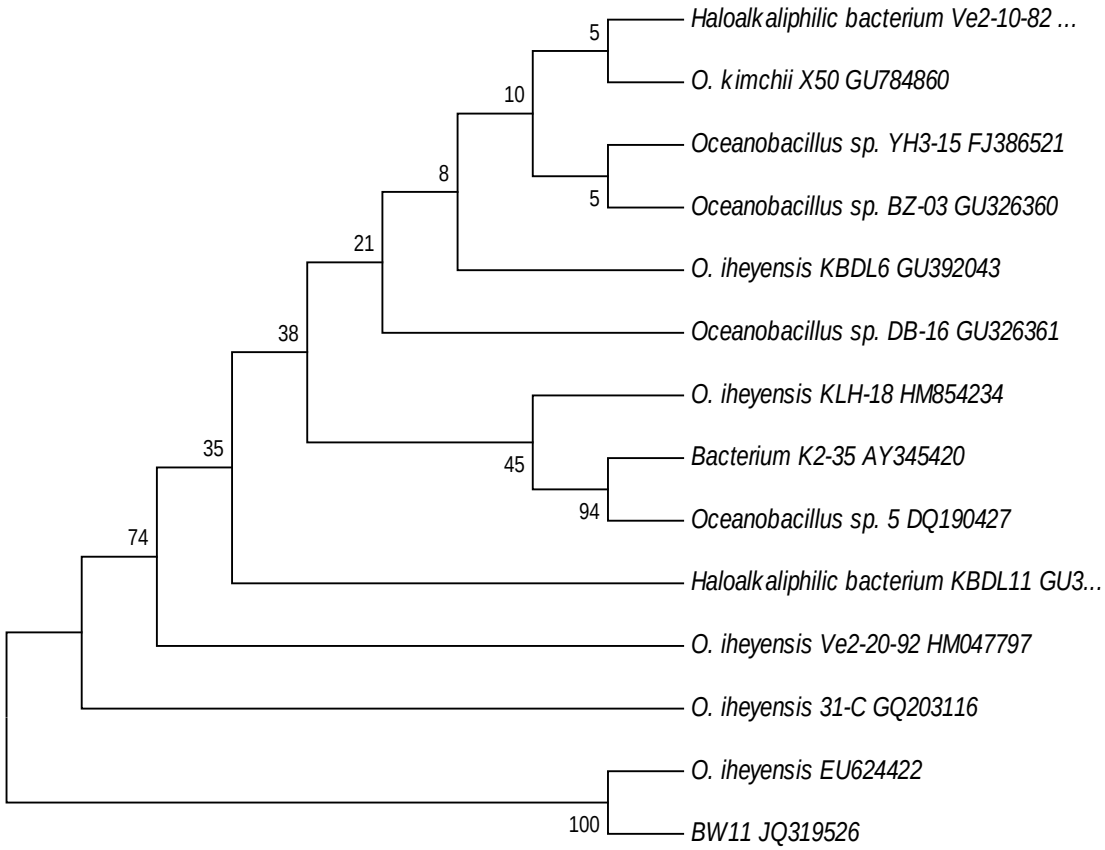


Fig. 1: Phylogenetic tree based on a comparison of the 16S ribosomal DNA sequences of Lonar lake isolates and some of their closest phylogenetic relatives

Table 2: Effect of Temperature and pH on antibacterial substance produced by *O. iheyensis*

		E. coli	*S. aureus*	*S. typhi*	*K. pneumoniae*	*P. vulgaris*	*E. aerogenes*	*P. aeruginosa*
Effect of Temperature on antibacterial substance	37ºC	+	+	+	+	-	-	-
	60ºC	+	-	-	+	-	-	-
	70ºC	+	-	-	+	-	-	-
	80ºC	+	-	-	+	-	-	-
	90ºC	-	-	-	+	-	-	-
	100ºC	-	-	-	+	-	-	-
	121ºC	-	-	-	+	-	-	-
Effect of pH on antibacterial substance	12	+	+	+	+	-	-	-
	10	+	+	+	+	-	-	-
	9	+	-	-	+	-	-	-
	8	+	-	-	+	-	-	-
	7	+	-	-	+	-	-	-

Fig 2: Effect of temperature on antibacterial substnace

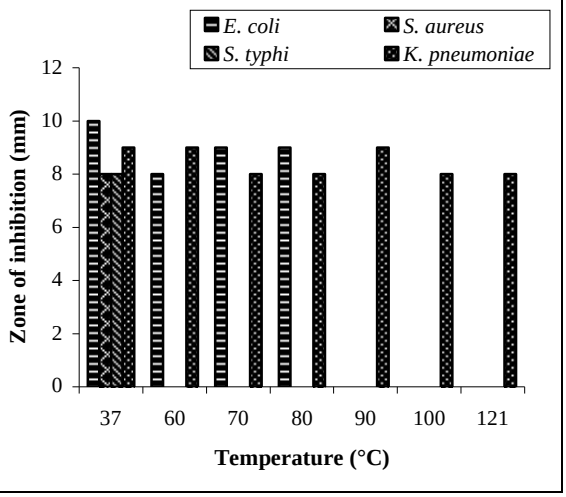
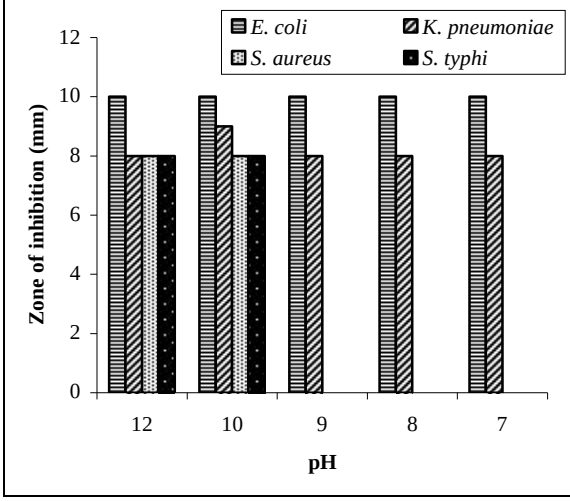


Fig 3: Effect of pH on antibacterial substance



CONCLUSION

Our study revealed haloalkaliphilic *Oceanobacillus* in a Lonar lake, and these kinds of bacteria could produce broad spectrum of antimicrobial agents. However, further studies are needed to identify the components of the *Oceanobacillus* responsible for the biological activity. These bacteria in general represent a new and rich source of secondary metabolites that need to be explored. The developing novel techniques in genetic engineering combined with better knowledge of structure and function allow fulfillment of inhibitory activity against pathogenic bacteria and exploration of novel applications in pharmaceutical. The genetic characterization of strain *O. iheyensis* will encourage future application of genetic methods toward strain development for improved their antibacterial activity.

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