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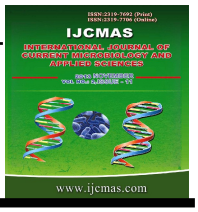

Original Research Article

Isolation, Identification and Characterization of *Pseudomonas* species from Lonar lake for Production of Lipase

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A B S T R A C T

Thirty seven cultures were isolated from soil & water samples collected from Lonar crater lake situated in Buldhana district, Maharashtra state, India. Primarily the cultures were isolated by using Nutrient media, HoriKoshi I & II media & finally Selective media like *Pseudomonas* isolation agar were used & thirty seven cultures were isolated. These were identified as haloalkaliphilc *Pseudomonas* species by using morphological, physiological & biochemical methods. Out of these, three isolates were found to have growth in the pH range from pH 7 to 11 & in the temperature range of 10 to 55°C. These three cultures were screened for lipase production & one culture showed largest zone of hydrolysis on Tween 80 assay media plate. This culture was named as PS (*Pseudomonas* species)L1 & 16s RNA sequence analysis of the same confirmed that it is *Pseudomonas* species. It will be later used for production of Lipase.

K e y w o r d s

Pseudomonas; halo-alkaliphilic; lipase; Lonar crater

Introduction

Microbes are ubiquitus in nature on earth. *Pseudomonas* species are one of the bacterial species found on earth. The habitats for growth of *Pseudomonas* sp. include normal soil, water, air. But apart from these, *Pseudomonas* sp. are also inhabitant of some extreme environments on earth[3] (Horikoshi et al. 1982) like hypersaline lakes[3,8] (Horikoshi et al. 1982, Ulukanly et al. 2002) dead sea & volcanic acid lakes. One of these extreme habitats is Lonar crater soda lake. The pH of the Lonar soda lake is alkaline having approximate range in between pH 8- 14. The pH is higher during summer season. These *Pseudomonas* sp. are capable of producing different industrially important enzymes. Lipases (E.C.3.1.1.3) are one of them that are currently used in different industries like detergent industries, leather industries, chemical industries, pharmaceutical industries, etc. If the microbial species is isolated from extreme environment the enzymes produced by that species may be more stable & may remain active at different reaction stages of varying physiological conditions during industrial processes (Sharma *et al*., 2001).

Materials and Methods

Sample collection

The soil & water samples are collected from Lonar crater lake(Tambekar *et al.*, 2010, Tambekar *et al*., 2012)The soil samples are collected in sterile plastic zipper (polythene) bags by digging the Lake shore 5-10 cm deep from different sites around the lake & rhizospheric soil of of some inhabitant plants also collected with sterile spatula[4].(Joshi et al. 2007). The pH & temperature of the lake water was recorded in March 28, 2011. The pH was 9.5 and temperature was 27°C. Water sample I & II are collected in sterile water Sampling bottles from different sites around the shore. Both soil & water samples are kept in an icepack cabinet maintained at temperature below 10°C.

Media used

The different media used for isolation & identification were Nutrient broth & agar, Horikoshi media I & II broth & agar[3] (Horikoshi et al. 1982), cetrimide broth & agar and selective media include *Pseudomonas* isolation agar base, *Pseudomonas* agar for fluorescein, some of which compositions & specifications are given below: Horikoshi I & II media (Horikoshi *et al*., 1982), (For isolation of alkaliphilic microbes)
Horikoshi medium

Isolation of Bacterial species

The collected water samples were added in Nutrient broth of pH 7, 9.5 & 11 within 6 hrs. of sampling & enriched by incubating them at optimum temperature for 18 to 24 hrs in a rotary Shaker at 120 rpm at 27°C at Microbiology lab, Dept. of Microbiology, Yeshwant College, Nanded, Maharashtra, India. After incubation the enriched mixed culture from water samples were streaked on to Nutrient agar plates, Cetrimide agar plates, *Pseudomonas* isolation agar plates of pH 7 , 9.5 & 11 , Horikoshi I & II agar plates and incubated at optimum temperature for 18 to 24 hrs. The isolated colonies observed after incubation & colony characters were recorded were then subcultured on to respective media slants of respective pH. The soil samples from lake shore & from rhizosphere of some inhabitant plants were serially diluted by using sterile D/W & higher dilutions were spreaded on to Nutrient agar plates, Cetrimide agar plates, *Pseudomonas* isolation agar plates of pH 7 , 9.5 & 11 , Horikoshi I & II agar plates and incubated at optimum temperature for 18 to 24 hrs. The isolated colonies observed after incubation & colony characters were recorded were then subcultured on to respective media slants of respective pH.

Results and Discussion

Identification of *Pseudomonas* species

The *Pseudomonas* species were identified by performing different biochemical tests on the isolated cultures & were confirmed by using Bergys manual of determinative bacteriology[1](Bergey, D.H., 1939). The performed biochemical tests & Grams staining results were as shown in table.

Screening of Selected Cultures for Production of Lipase on Tween-80 & Egg yolk agar medium

After recording biochemical test results most of the culures were identified as

>UI23_Assambled_Sequence

CCGAAAAGCCCGGGAACAGGAAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGA
CGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA
CCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACG
GTTTCGGCTGTCACTTACAGATGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAA
TGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG
CAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGA
TCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGAGGTAACTGCTCGCACCTT
GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGG
TTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAA
TGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAC
TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGT
CCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTG
CAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAG
CA ACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAAC

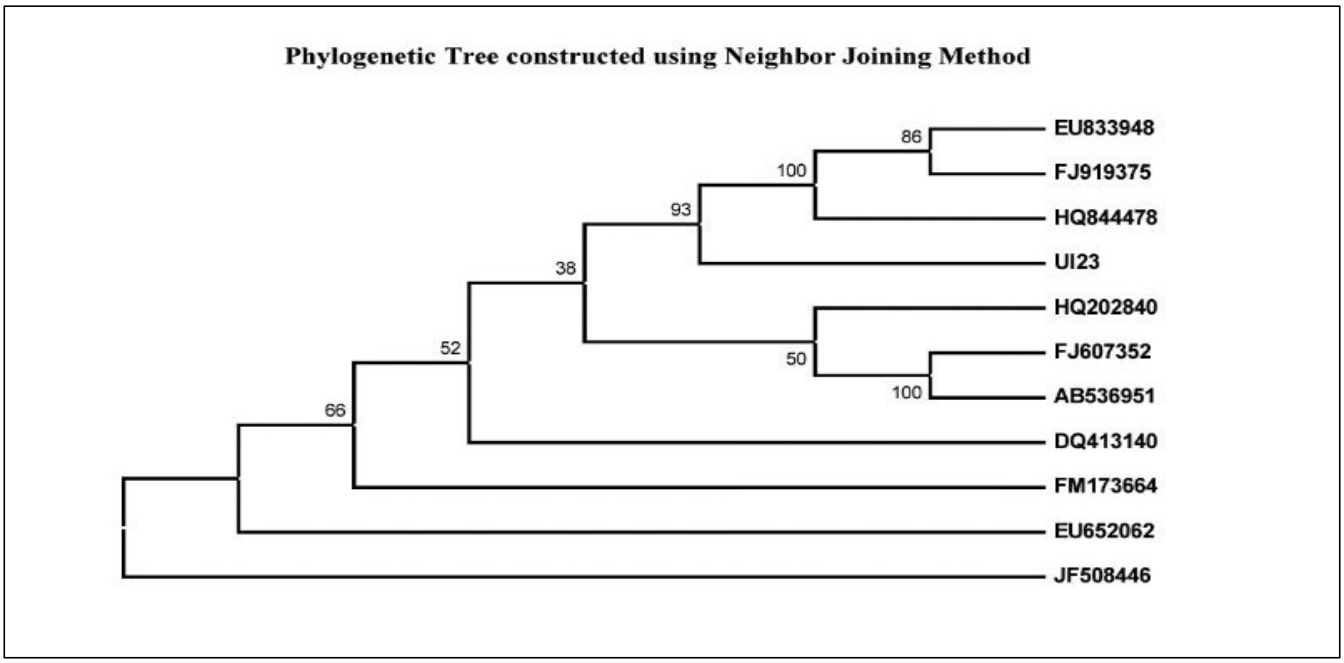
Distance Matrix based on Nucleotide Sequence Homology

Accession ID		1	2	3	4	5	6	7	8	9	10	11
UI23	1	---	0.009	0.007	0.009	0.011	0.011	0.012	0.007	0.013	0.006	0.021
FJ607352	2	0.053	---	0.010	0.010	0.012	0.013	0.013	0.009	0.010	0.009	0.022
HQ844478	3	0.038	0.073	---	0.010	0.012	0.011	0.013	0.003	0.014	0.003	0.022
FM173664	4	0.072	0.083	0.072	---	0.011	0.010	0.013	0.010	0.015	0.009	0.022
HQ202840	5	0.091	0.098	0.103	0.097	---	0.013	0.013	0.012	0.015	0.012	0.023
EU652062	6	0.083	0.110	0.098	0.083	0.130	---	0.014	0.011	0.017	0.011	0.020
DQ413140	7	0.094	0.118	0.123	0.116	0.129	0.148	---	0.013	0.017	0.013	0.024
EU833948	8	0.036	0.063	0.011	0.066	0.101	0.098	0.113	---	0.014	0.002	0.022
AB536951	9	0.130	0.075	0.149	0.161	0.158	0.187	0.201	0.141	---	0.014	0.025
FJ919375	10	0.034	0.066	0.011	0.066	0.104	0.098	0.113	0.003	0.145	---	0.022
JF508446	11	0.281	0.302	0.298	0.296	0.330	0.290	0.318	0.294	0.370	0.292	---

Table indicates nucleotide similarity (above diagonal) and distance (below diagonal) identities between the studied sample **UI23** and ten other closest homologs microbe

Sr. NO.	Test	Grams staining	Catalase	Oxidase	Amylase	Gelatinase	Glucose	Lactose	PHB	Indole	MR	VP	Citrate	Tween 80 hydrolysis	Lecithinase	Xylose	D-Arabinose	Mannitol	Lipase
1	PSL4	-	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-	+	-
2	PSLF3	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-
3	PSL8P1	-	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+	+
4	PSLF5	-	+	+	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-
5	PSL21P2	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+
6	PSL13P1	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+
7	PSLF1	-	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	+	-
8	PSL7H	-	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	+
9	PSL7P1	-	+	+	+	+	-	-	-	-	-	+	+	d	+	-	-	+	+
10	PSL13H	-	+	+	+	+	+	-	-	-	-	-	+	d	+	-	-	+	+
11	PSL21H	-	+	+	+	+	+	-	-	-	-	-	+	d	-	-	-	-	+
12	PSLF2	-	+	+	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+
13	PSL8P2	-	+	+	+	+	+	-	-	-	-	-	+	d	+	-	-	-	+
14	PSLMUM1	-	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
15	PSL41	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
16	PSL42	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-
17	PSL48	-	+	+	-	+	-	-	-	-	-	-	+	+	+	-	-	+	+
18	PSL46	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	-	+	+
19	PSL98	-	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	-
20	PSL58	-	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-
21	PSL42H	-	+	+	-	+	-	-	+	-	-	+	+	-	-	-	-	+	-

22	PSL97	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+	-
23	PSL1	-	+	+	-	+	-	-	-	-	-	-	+	+	+	-	-	+	+
24	PSL63	-	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-	+	-
25	PSL90	-	+	+	-	+	+	-	-	-	-	+	+	+	-	-	-	+	-
26	PSL91	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+
27	PSL69	-	+	+	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+
28	PSL64	-	+	d	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+
29	PSL76	-	+	+	-	+	+	-	+	-	-	+	+	+	-	-	-	+	-
30	PSL77	-	+	+	-	+	-	-	-	-	-	+	+	+	-	-	-	+	-
31	PSL81	-	+	+	-	+	-	-	-	-	-	+	-	+	+	-	-	+	+
32	PSL86	-	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+
33	PSL83	-	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-
34	PSL80	-	+	+	-	+	-	-	+	-	-	-	+	+	+	-	-	+	+
35	PSL75	-	+	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
36	PSLS1Y	-	+	+	+	+	+	-	-	-	-	-	+	-	+	-	'	+	+
37	PSL B 10	-	+	+	-	+	+	-	-	-	-	-	+	-	+	-	-	-	+



Pseudomonas species. Three cultures were showing highest zone of Tween 80 hydrolysis (Dahiya *et al.*, 2011) & highest zone on Egg yolk medium respectively. These were chosen & one out of these that was no. 23 culture (PSL1) was again confirmed by 16s RNA sequencing and bioinformatic analysis (Tambekar *et al.*, 2012) as Pseudomonas species L1 results of which are given .

Steps for 16s r-RNA analysis were as follows

1.PCR amplification of genomic DNA with universal primers specific for 16S rRNA amplification.

2.The PCR product was bi-directionally sequenced using 16S specific primers.

3.Sequence data was aligned and analyzed for finding the closest homologs for the sample. Based on nucleotides homology and phylogenetic analysis the sample UI23 was detected to be *Pseudomonas aeruginosa* (Accession No: HQ844478). Nearest homolog species was found to be *Pseudomonas xanthomarina* (Accession No. HQ202840). From the above results it was confirmed that the thirty seven bacterial isolates from Lonar lake were alkaliphilic *Pseudomonas sp.* & are capable of producing lipase enzyme will be used for production of lipase.

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