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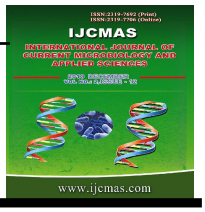

Original Research Article

Isolation, Identification and Characterization of Bacillus species from Lonar lake for production of Cyclodextrin Glycosyltransferase

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Keywords

Aerolite Meteor; Halo-alkaliphilic; Bacillus; Cyclodextrin Glycosyl-transferase (CGTase).

A B S T R A C T

The Lonar crater has been proved to be caused by an Aerolite Meteor (contain mainly rocky material) because no metal fragments have been found strewn around the crater. The pH values generally higher than 10 and occasionally reaching 12. The aim of the present study was to isolate Fourty five cultures from soil & water samples collected from Lonar crater lake situated in Dist. Buldhana, Maharashtra, India. Primarily the cultures were isolated by using Nutrient media. Horikoshi II media & finally selective media like Hichrome Bacillus agar and Hichrome UTI agar modified were used for the isolation of fourty five cultures. These were identified as alkaliphilic Bacillus species by using morphological, physiological& biochemical methods. Out of these five isolates were found to have growth in the pH range from pH 7 to 11 & in the temperature range of 15 to 45^{0}C. These Five cultures were screened for Cyclodextrin Glycosyltransferase (CGTase) production & two cultures showed largest zone of hydrolysis on Modified Horikoshi II assay media plate containing phenolphthalein indicator and methyl orange indicator stain. These cultures were named as BI33 and BI56A & 16s RNA sequence analysis of the same confirmed that these cultures were of Bacillus species. These cultures will be later used for CGTase production.

Introduction

In addition to their rich biodiversity, soda lakes often harbour many unique species, adapted to alkali conditions and unable to live in environments with neutral pH. These are called *alkaliphiles*. Organisms also adapted high salinity are called *haloaklaiphiles*. Culture-independent genetic surveys have shown that soda lakes contain an unusually high amount of alkaliphilic microorganisms with low genetic similarity to known species (Surakasi, 2010; Dong, 2006; Xiong, 2012; Wani, 2006).This indicates a long evolutionary history of adaptation to these habitats with few new species from other environments becoming adapted over time. Microbial communities in natural alkaline environments such as lakes have

attracted attention because of possible biotechnological use of enzyme and metabolites from such organisms. In a sense, extreme alkaline and Extremophiles, in general, are specialists since they have to be able to thrive under such harsh conditions. The pH of these lakes is higher during summer season. The habitat of Bacillus species include normal soil, water, air but including thses environments Bacillus species are also able to thrive in some extreme environments like soda lake (Horikoshi *et al.,* 1982).

Studies on alkaline enzymes have concentrated largely on those organisms which have been easily observed in the natural environment. For example, large numbers of alkaliphilic Bacillus species have been isolated over the years, many due to the systematic work of Horikoshi and co-workers. Archaeal isolates have also been targeted for exploration. Their chemistry is distinct and they contain such lipids, where their stereo-configuration is different to that in bacteria (Grant *et al*., 1990). Today, these organism are of considerable industrial interest, particularly for the production of enzymes cyclodextrin glucanotyransferase (E.C. 2.4.1.19) for cyclodextrin manufacture from starch, frequently used in foodstuffs, chemicals, cosmetics and pharmaceuticals (Grant et al., 1990; Horikoshi, 1996). The Bacillus species is capable of producing different industrially important enzymes.

Cyclodextrin Glycosyltransferases (E.C. 2.4.1.19) are one of the currently used enzyme in different industries for Cyclodextrin production, which have different applications in Stabilization of volatile materials (Flavors and spices), Deodorization of medicines and foods, etc. (Kitahata and Okada, 1974a; Kitahata *et al.* , 1974b). if the microbial species is isolated from extreme environment the enzyme produced by that species may be more stable & may remain active at different reaction stages of varying physiological conditions during industrial processes (Salva et al., 1997). Therefore it was thought to undertake studies on Isolation, Identification & characterization of Bacilllus species from lonar crater having pH values generally greater than 10 and some times 12 for production of CGTase.

Materials and Methods

The soil & water samples are collected from lonar crater lake (Tambekar *et al.*, 2010). 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 some inhabitant plants also collected with sterile spatula (Joshi *et al*., 2007; Watanabe and Hayano, 1993; Chilcott and Wigley, 1993). The pH & temperature of the lake water was recorded in march -2011. The pH was 9.5 and temperauture was 27^0C. water sample I and 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 10^0C.

Media Used

The different media used for isolateion & identificatipon were Nutrient broth & agar, Horikoshi media II broth & agar (Horikoshi *et al*., 1982), Modified Horikoshi media II with phenolphthalein indicator and Methyl orange stain (Salva *et al*., 1997). And selective media include Hichrome Bacillus agar and Hichrome UTI agar.

Isolation of Bacterial species

The collected soil samples from lake shore & from rhizosphere of inhabitant plants were serially diluted using sterile distilled water. Then diluted soil sample & water sample were added in nutrient broth of pH 7, 9.5 & 11 separately within 6 hrs of sampling and enriched by incubating them at optimum temperature for 18 to 24 hrs in a rotary shaker at 120 rpm at 30^0C at Research lab, Department of Microbiology, Yeshwant Mahavidyalaya, Nanded, Maharashtra, India.

After incubation the enriched samples were streaked on nutrient agar plate, Horikoshi media II agar plates, Horikoshi media II with phenolphthalein indicator and Methyl orange stain plates (Salva *et al*., 1997) of pH 7, 9.5, 11 and incubated at optimum temperature i.e. 30^0C for 18 to 24 hrs. the isolated colonies observed after incubation and colony characters were recorded. Then colonies were sub-cultured on to respective media slants of respective pH.

Identification of Bacterial species

The subcultured cultures on slants were used for identification of cultures using screening for CGTase production, biochemical analysis using Bergey's manual and 16S rRNA sequencing and bioinformatic analysis.

Results and Discussion

Fourty five cultures were isolated form lonar lake. Out of these isolates most isolates were identified as Bacillus species by performing different biochemical tests on the isolates and were confirmed by using Bergey's manual of determinative bacteriology (Beregy, 1939) (Table 1). Out of these isolates five isolates showed growth in pH range 7 to 11 and in temperature range 10 to 45°C. So, the selected five isolates were screened for CGTase production using Modified Horikoshi II assay media plate containing phenolphthalein indicator and methyl orange indicator stain as indicator of starch hydrolysis and β-cyclodextrin production. five cultures were screened and out of these five cultures only two cultures showed largest zone of hydrolysis on Modified Horikoshi II assay media plate (Salva *et al*., 1997). These isolates were selected and confirmed again by 16S rRNA sequencing (Table 2 & Table 3) and bioinfoematic analysis (Heyndrickx *et al*., 2004) as Bacillus species.

Steps for 16S rRNA analysis were as follows

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

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

Seqence data was aligned and analysed for finding the closest homologs for the sample. Based on nucleotide homology and phylogenetic analysis the sample BI56A was detected to be Bacillus flexux (Accession No.: JX 419382) Nearest homolog species was found to be Bacillus flexux (Accession No.: EU073093) (Figure 1 & Figure2).

Based on nucleotide homology and phylogenetic analysis the sample BI33 was detected to be Bacillus species (Accession No.: JX 419381) Nearest homolog species was found to be Bacillus species (Accession No.: AAXV01000001) (Figure 3 & Figure 4).

Table.1 Biochemical Analysis of the Bacterial Isolates

Sr. No	Test	Endospore	Gram nature	Catalase	Oxidase	Amylase	Gelatinase	Urease	Indole	VP	MR	Citrate	Glucose	Xylose	Mannitol	Lecithinase	Growth at 45^0C	Growth at 65^0C
1	BI01	Central	+	+	+	+	-	-	-	+	-	+	+	+	-	-	+	+
2	BI02	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+
3	BI03	Central	+	+	+	+	+	-	-	+	-	+	-	+	+	-	+	-
4	BI04	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
5	BI05	Central	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-
6	BI06	Central	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	-
7	BI09	Central	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
8	BI10	Central	+	+	+	+	+	-	-	+	-	+	-	+	+	-	+	-
9	BI11	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
10	BI33	Central	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-
11	BI34	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
12	BI35	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
13	BI36	Central	+	+	+	-	+	-	-	+	-	-	+	+	+	-	+	+
14	BI37	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+
15	BI38	Central	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	+
16	BI39	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
17	BI40	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
18	BI41	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-
19	BI42	Central	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	-
20	BI43	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-

21	BI44	Central	+	+	+	+	+	+	-	+	-	+	+	-	+	-	-	-
22	BI22A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+
23	BI23A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+
24	BI24A	Central	+	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+
25	BI25A	Central	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-
26	BI26A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-
27	BI27A	Central	+	+	D*	+	+	-	-	+	-	+	+	+	-	-	+	+
28	BI28A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
29	BI30A	Central	+	+	+	+	-	-	-	+	-	-	+	+	+	-	+	-
30	BI31A	Central	+	+	+	+	+	-	+	+	-	+	+	+	-	-	-	-
31	BI32A	Central	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-
32	BI33A	Central	+	+	+	-	+	-	-	-	-	+	+	-	+	-	-	-
33	BI34A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
34	BI35A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
35	BI36A	Central	+	+	+	+	+	-	-	+	-	+	-	+	+	-	+	-
36	BI37A	Central	+	+	+	+	+	-	-	+	-	+	-	-	+	+	+	-
37	BI43A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
38	BI44A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
39	BI45A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+
40	BI47A	Central	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+
41	BI51A	Central	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+
42	BI54A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
43	BI56A	Central	+	+	+	+	+	-	-	+	-	+	+	+	+	-	+	-
44	BI62A	Central	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+
45	BI66A	Central	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+

***('+' means Positive; '-' Means Negative; 'D' means positive between 11- 89%)**

Table.2 BI56A Assembled sequence

>B156A_ Assembled sequence

GACCTTTGCCGGCTTGCCTAATGATTGCAAGTCGAGCGAACTGATTAGAAGCTTG
CTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTA
AGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAACATTTTCTCTTG
CATAAGAGAAAATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGG
TGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGA
CCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC
CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACA
AGTACAAGAGTAACTGCTTGTACCTTGACGGTACCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATT
ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAA
AGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGT
GGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGCTAAGTG
TTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGG
GGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
ACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTAAGTTGGGCACTCTAAGGTG
ACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGAC
CGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCA
ACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAAC
ACCCGAAGTCGGTGGGGTAACCTTTATGGAGCCAGCCGCCTAAGGGGGACAAAA
TTTGG

Table.3 BI33 Assembled sequence

>BI33_16S-1464 bases

CGGGCGGGGTGCCCTTATTACATGCAAGTCGAGCGAACAGAGAAGGAGCTTGCT
CCTTCGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTACCTTATAG
TTTGGGATAACTCCGGGAAACCGGGGCTAATACCGAATAATCTGTTTCACCTCAT
GGTGAAACACTGAAAGACGGTTTCGGCTGTCGCTATAGGATGGGCCCGCGGCGC
ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGC
GTGAGTGAAGAAGGATTTCGGTTCGTAAAACTCTGTTGTAAGGGAAGAACAAGT
ACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACT

ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTG
GGCGTAAAGCGCGCGCAGGTGTTTTCTAAGTCTGATGTGAAAGCCCACGGGCTC
AACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGATAGTG
GAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTTGGAGGAACACCAGTGGC
GAAGGCGACTATCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT
AGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG
AGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC
ATCCCGTTGACCACTGTAGAGATATGGTTTCCCCTTCGGGGGCAACGGTGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA
TGACCTGGGCTACACACGTGCTACAATGGACGATACAAACGGTTGCCAACTCGC
GAGAGGGAGCTAATCCGATAAAGTCGTTCTCAGTTCGGATTGTAGGCTGCAACT
CGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAA
TACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACC
CGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCGCCGAAAGGGTGGAATAGAAA
GT

Figure.1 Distance Matrix based on Nucleotide Sequence Homology of BI56A

Distance Matrix based on Nucleotide Sequence Homology

		1	2	3	4	5	6	7	8	9	10	11
B156A	1	—	0.002	0.002	0.000	0.000	0.002	0.004	0.005	0.002	0.000	0.004
AB274759	2	0.002	—	0.001	0.002	0.001	0.001	0.000	0.002	0.002	0.000	0.001
AB366310	3	0.002	0.001	—	0.002	0.001	0.001	0.001	0.001	0.003	0.001	0.001
EU073093	4	0.000	0.002	0.002	—	0.000	0.002	0.004	0.005	0.002	0.000	0.004
EU867379	5	0.000	0.001	0.001	0.000	—	0.001	0.001	0.000	0.000	0.000	0.001
HM451441	6	0.002	0.001	0.001	0.002	0.001	—	0.000	0.002	0.002	0.000	0.000
HM566047	7	0.004	0.000	0.001	0.004	0.001	0.000	—	0.001	0.004	0.000	0.002
HM566050	8	0.005	0.002	0.001	0.005	0.000	0.002	0.001	—	0.003	0.000	0.004
HQ234345	9	0.002	0.002	0.003	0.002	0.000	0.002	0.004	0.003	—	0.000	0.005
HQ678679	10	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	—	0.000
HQ908674	11	0.004	0.001	0.001	0.004	0.001	0.000	0.002	0.004	0.005	0.000	—

Table indicates nucleotide similarity (above diagonal) and distance (below diagonal) identities between the studied sample **BI56A** and ten other closest homologs microbe.

Figure.2 Phylogenetic Tree constructed using Neighbor Joining Method of BI56A

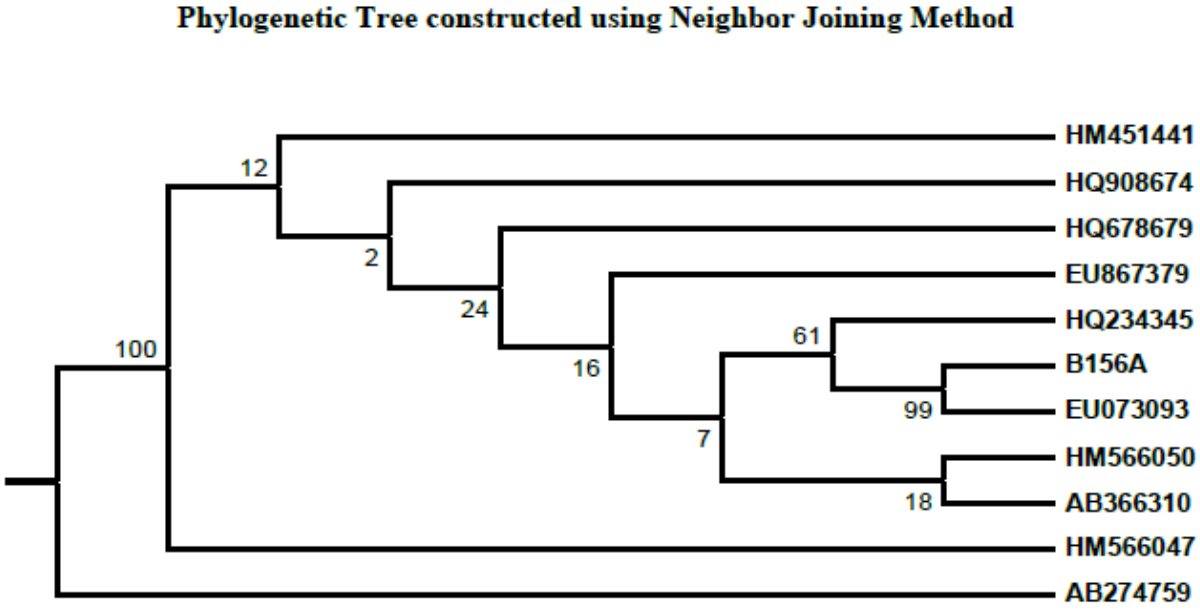


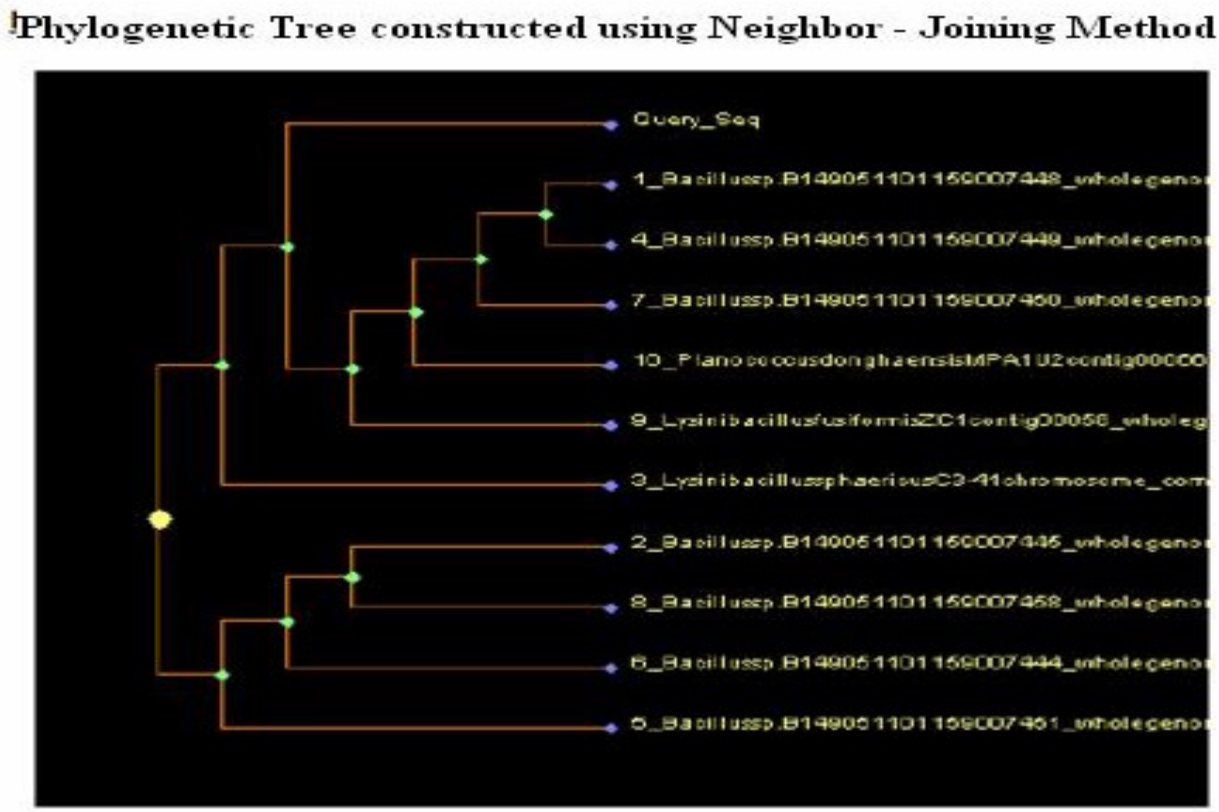
Figure.3 Distance Matrix based on Nucleotide Sequence Homology of BI33

Distance Matrix based on Nucleotide Sequence Homology

2_Bacillus	-	0	0	0	0	0	0.01	1.27	1.26	1.26	1.3
8_Bacillus	0	-	0	0	0.01	0.01	0.02	1.25	1.24	1.24	1.28
6_Bacillus	0	0	-	0	0	0.01	0.02	1.28	1.27	1.27	1.3
5_Bacillus	0	0	0	-	0	0	0.01	1.27	1.27	1.26	1.3
3_Bacillus	0	0.01	0	0	-	0.01	0.01	1.27	1.27	1.27	1.3
Query_Seq	0	0.01	0.01	0	0.01	-	0.02	1.29	1.29	1.28	1.31
9_Lysinibacillus	0.01	0.02	0.02	0.01	0.01	0.02	-	1.28	1.27	1.28	1.31
1_Bacillus	1.27	1.25	1.28	1.27	1.27	1.29	1.28	-	0	0.01	0.07
4_Bacillus	1.26	1.24	1.27	1.27	1.27	1.29	1.27	0	-	0.01	0.07
7_Bacillus	1.26	1.24	1.27	1.26	1.27	1.28	1.28	0.01	0.01	-	0.07
10_Planoco	1.3	1.28	1.3	1.3	1.3	1.31	1.31	0.07	0.07	0.07	-

Table indicates nucleotide similarity (above diagonal) and distance (below diagonal) identities between the studied sample BI33 and ten other closest homologs microbe.

Figure.4 Phylogenetic Tree constructed using Neighbor Joining Method of BI33



From the above result it was confirmed that the fourty five bacterial isolates from lonar lake were alkaliphilic pseudomonas species and are capable of producing Cyclodextrin Glycosyltransferase (CGTase) enzyme & will be used for production of Cyclodextrin Glycosyltransferase (CGTase).

References

Bergey, D.H., 1939. Manual of determinative bacteriology. Williams and Wilkins, Baltimore . 5th Ed.

Chilcott C.N., and Wigley, P.J. 1993. Isolation and toxicity of Bacillus thuringiensis from soil and insect habitats in New Zealand. J. Inverteb. Path. 61: 244-247.

Dong, H., G. Zhang, H. Jiang, B.Yu, L.R. Chapman, C.R. Lucas and Fields M. W. 2006. Microbial Diversity in Sediments of Saline Qinghai Lake, China: Linking Geochemical Controls to Microbial Ecology. Microbial Ecol.51 (1): 65–82.

Grant, W.D., W.E. Mwatha and Jones, B.E. 1990 Alkaliphiles : ecology, diversity & applications. FEMS Microbiol. Rev. 75, 255-270

Heyndrickx, M., N.A. Logan, L. Lebbe, M. Rodriguez-Diaz, G. Forsyth, J. Goris,P. Scheldeman and De Vos, P. 2004. Bacillus galactosidilyticus sp. nov., an alkali-tolerant, β-galactosidase producer. Int. J. Syst. Evol. Microbiol. 54: 617–621.

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

Horikoshi, K., and Akiba, T. 1982. Alkalophilic microorganisms: a new microbial world. Springer, New York.

Joshi A. A., P.P. Kanekar, A.S. Kelkar, Y.S., Shouche , A.A. Wani, S.B. Borgave and Sarnaik. 2007. Cultivable bacterial diversity of Alkaline Lonar Lake, India. Microb. Ecol. 55: 163-172.

Kitahata, M., and Okada, S. 1974a. Action of Cyclodextrin glycosyltransferase from Bacillus megaterium strain No. 5 on starch. Agric. Biol. Chem. 38: 2413-2417

Kitahata, S., N. Tsuyama and Okada, S. 1974b. Purification and some properties of Cyclodextrin glycosyltransferase from a strain of *Bacillus* species. Agric. Biol. Chem. 38: 387-393.

Salva, T. J. G., V. Bittencourt de Lima and Pagan, A.P. 1997. Screening of alkalophilic bacteria for cyclodextrin glycosyltransferase production. Rev. Microbiol. 28: 157-164.

Surakasi, V. P., C.P. Antony, S. Sharma, M.S. Patole and Shouche Y. S. 2010. Temporal bacterial diversity and detection of putative methanotrophs in surface mats of Lonar crater lake. J. Basic Microbiol. 50 (5): 465–474.

Tambekar, D. H., A.L. Pawar and Dudhane M. N. 2010. Lonar Lake water: Past and Present. Nat. Environ. Poll. Technol**.** 9(2): 217-221.

Wani, A. A., V.P. Surakasi, J. Siddharth, R.G. Raghavan, M.S. Patole, D. Ranade and Shouche Y. S. 2006. Molecular analyses of microbial diversity associated with the Lonar soda lake in India: An impact crater in a basalt area. Res. Microbiol.157 (10): 928–937.

Watanabe, K., and Hayano, K. 1993. Distribution and identification of proteolytic Bacillus spp. in paddy field soil under rice cultivation. Can. J. Microbiol. 39: 674-680.

Xiong, J., Y. Liu, X. Lin, H. Zhang, J. Zeng , J. Hou, Y. Yang and Yao, T. 2012. Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan Plateau. Environ. Microbiol. 14(9):2457-2466