Isolation and Identification of Actinobacteria (*Kocuria sp., Conexibacter sp., Brevibacterium sp. and Rubrobacter sp.*) from Lonar lake District Buldhana, Maharashtra.

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***Abstract*- During the study out of 60 water samples 04 actinomycete isolates were obtained which were labelled from ACM1 to ACM 4. All samples are collected from the five different sample collection sites from the Lonar lake area were selected for the collection of water samples. Such as Dhar water spot, Ramgaya Spot, Kamalaji Devi Temple spot, Ambarkhana Mahadev Temple spot, Mungala Mahadev Temple spot. The present study was performed by collection and examination of Lonar Lake water samples during three different seasons. Samples were collected in sterile bottles and later transferred to P. G. Department of Microbiology, Shri Shivaji Science and Arts College, Chikhli, Dist. Buldhana for further study. It includes Physicochemical Analysis of water, Salinity (By using Chloride content), Isolation of diversity of Actinomycetes from water and sediment samples of Lonar Crater, Characterization and identification of Actinobacteria, Identification of actinobacteria on the basis of 16s RNA, Extraction of genomic DNA Phylogenic analysis of actinomycetes. The genomic DNA of 4 Actinomycetes isolates was extracted by using a commercially available kit according to the manufacturer's guide. We isolates four actinomycete (*Kocuria* sp., *Conexibacter* sp., *Brevibacterium* sp. and *Rubrobacter* sp.) and complete his further study. We have received accession numbers for all the sequences. The isolated actinomycetes species were ACM 01 *Kocuria rhizophila* (ON248525), ACM 02 *Conexibacter woesei* (ON377276), ACM03 *Brevibacterium spp*., ACM 04 *Rubrobacter xylanophilus* (ON387757).**

***Index Terms*- Actinomycetes, *Kocuria* sp., *Conexibacter* sp., *Brevibacterium* sp. and *Rubrobacter* sp.,**

I. INTRODUCTION

Actinomycetes are fungus-like filamentous bacteria that are gram-positive and facultatively anaerobic. They are the top natural antibiotic producers (Bhatti *et al.,*2017). Actinomycetes are the most economically valuable prokaryotes which are well known to produce chemically diverse metabolites with wide range of biological activity (Balagurunathan *et al*., 2007). About 90% of Actinomycetes genera have been isolated from soil, and they are safe for use in a variety of industries, including the industrial and medicinal sectors (Bawazir *et al.,*2018). Actinomycetes are predominantly Gram-positive, aerobic, heterotrophic, and non-motile. Colonies of Actinomycetes usually have a firm consistency and adhere tenaciously to the solidified substratum. In certain Actinomycetes genera, the surface appears powdery and often becomes pigmented when dying aerial spores are produced. Their ubiquitous nature, different structure, the ability for the production of secondary metabolites and enzymes makes them one of the most important groups of organisms distinct from other bacterial species. Some Actinomycetes are pathogenic to man, domestic animals, and a few plants such as potatoes, whereas some are in a mutualistic association with plants and animals (Goodfellow *et al.,* 2012). Actinomycetes, high guanine and cytosine- containing bacteria are one of the most dominating phyla of the bacteria found on almost all natural substrates (Rakshanya *et al.,* 2011).

Size, shape, texture, colour consistency on diverse mediums and the presence or absence of aerial mycelia are all examples of colony features. Following that, as mentioned in the pigmentation studies in terms of colors, diffusive nature of pigments, and reversal of colonies (Shirling and Gottlieb, 1966) assist in identification. Using a reference color key, the color and pigmentation of substrate mycelia microscopic characteristics are noted. Gram's microscope was used to examine the colonies' microscopic characteristics. Coverslip culture and slide culture procedures are used to investigate staining (Gram, 1884).

Morphological and chemotaxonomic characterizations are valuable tools for identifying actinomycetes, but they may miss species from many genera with comparable morphological and chemotaxonomic characteristics (Wang *et al*. 1999; Zhang *et al.* 1998). Many species in practically all taxonomic ranks have had their taxonomic placements determined using polyphasic techniques, including the 16S rRNA gene sequence (Wang *et al*. 1999). The marine environment is an excellent source of alkali-halophilic Streptomyces with possibly novel bioactive chemicals that might be useful in future bioprospecting in the agricultural, industrial, and pharmaceutical sectors (Malviya *et al.,* 2014).

In general, numerous pretreatment methods were utilized to limit the development of bacteria and fungus, such as the integration of specialized antibiotics, and the composition of the culture medium was altered specifically for the isolation of uncommon actinomycetes. Humic acid with various quantities of vitamin agar medium, on the other hand, proved to be the finest foundation for the selection of uncommon halophilic actinomycetes. By limiting the synthesis of diffusible pigments in the growth medium, humic acid improves morphological identification and characterizes the spore chain of actinomycetes (Arasu *et al.,* 2016).

Nearly 23,000 bioactive compounds are known to be produced by micro-organisms and around 10,000 of these bioactive secondary metabolites are produced by actinomycetes alone accounting for almost 45% of the total discovered bioactive compounds. Out of the total 10, 000 almost 7, 600 secondary metabolites are produced by *Streptomyces* species (Tiwari *et al.,* 2019**).** Most of the bioactive compounds produced by actinomycetes are potential antibiotics that are exploited in the pharmaceutical industry. Actinomycetes are one of the most economically valuable prokaryotes that have numerous biotechnological applications due to the synthesis of a vast range of secondary metabolites like antibiotics, antimicrobial agents, antitumor agents and enzymes (Valli *et al.,* 2012**).** These bioactive compounds show potential antibacterial, antifungal, antihelmintic, anti-malarial and anti-inflammatory properties. Some of the important genera of actinomycetes include *Streptomyces*, *Thermonospora*, *Microbispora*, *Nocardia*, *Micromonospora*, *Kitasatospora*, *Actinomadura*, *Rhodococcus*, *Actinoplane*, *Actinopolyspora* are reported to produce broad-spectrum antibiotics. Antibiotics derived from Streptomyces species are used as antibacterial and antifungal agents including streptomycin produced by *S. griseus*, Neomycin produced by *S. fradiae*, Kanzmycin produced by *S. kanamyceticus*, Tetracyclin produced by *S. rimosus*, Chloramphenicol produced by *S. venezuelae*, antitumor drugs including Anthracyclins, peptides, Aurelic acids, Carzinophilin, Mitomycins, antimetabolites are also obtained from Streptomyces species **(**Ouchari *et al.,* 2019; Tiwari *et al.,* 2019**).**

Scientists throughout the world are working tirelessly to search for new antibiotics to deal with the ever-rising problem of increasing drug resistance in micro-organisms. To tackle this problem exploration of new habitats and isolation of actinomycetes for the search of novel bioactive secondary metabolites needs to be performed (Jagannathan *et al.,* 2021).

II. MATERIAL AND METHODS

Lonar ($19^0$58'N, $76^0$31'E) is situated in the Buldana district (Maharashtra, India) and is one of the India's youngest impact craters a formerly volcanic Deccan-Trap geological region. Five different sample collection sites from the Lonar lake area were selected for the collection of water samples. Such as Dhar water spot, Ramgaya Spot, Kamalaji Devi Temple spot, Ambarkhana Mahadev Temple spot, Mungala Mahadev Temple spot.

Sediments and water samples were collected from each sampling spot seasonally for a period 3 years from January 2015 to December 2018. Calendar year for the study was divided in to three season viz. Post Monsoon (October – March), summer (April – May) and Monsoon (June – September). The present study was performed by collection and examination of Lonar Lake water samples during three different seasons. Samples were collected in sterile bottles and later transferred to P. G. Department of Microbiology, Shri Shivaji Science and Arts College, Chikhli, Dist. Buldana.

1. Physicochemical Analysis of water:

a) Total Dissolved Solids: Follows the routine method and used the following formula to calculate the Total Dissolved Solids.

Calculation:

$$\text{TDS mg/L} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Volume of sample}} \times 10^{6}$$

b) Salinity (By using Chloride content):

Firstly Standardization of $AgNO_3$ by using standard procedure then estimation of chloride and calculate the salinity by using following formula.

Calculation of Chloride:

$$\text{Chloride mg/L} = \frac{\text{mL of titrant} \times 0.014 \times 1000 \times 35.5}{\text{Volume of Sample}}$$

Calculation of Salinity:

$$\text{Salinity (mg/L)} = 0.03 + 1.806 \times \text{Chloride in mg/L}$$

2. Isolation of diversity of Actinomycetes from water and sediment samples of Lonar Crater.

Methods :-

i. Inoculation:- For the inoculation, sample aliquots (100 µl) were taken with the help of a pipette and transferred on the surface actinomycetes isolation agar media and Kenknight and Munaier's medium containing plates. With the help of a flame sterilized bend glass rod spreading was performed immediately. Utmost care was taken to make gentle movements with the spreader. Further the plates were incubated at 30^0C for 21 days with very 24 hr monitoring.

ii. Purification of isolates

After growth was noticed on the plates, colonies depicting typical actinomycetes morphology were picked up using sterilized nichrome wire loop and streaked on the same media plates on which they were observed. The plates were incubated at 30ºC.

3. Characterization and identification of Actinobacteria

Morphological Characterization was performed with a magnified lens. The actinobacteria strains were grown for 3 to 14 days on Actinomycetes Isolation agar plate. Colony morphology was recorded with respect to colour, aerial mycelium, and size, nature of colony, reverse side colour and pigmentation. A coverslip culture technique was adopted for light microscopic studies (Pridham *et al.,* 1958) Actinomycetes culture plate was prepared and 5 to 6 sterile cover slips were placed at an angle of 450. The actinomycetes culture was slowly released at the intersection of medium and cover slip. The plates were incubated at 28 +/ - 2 ^{0}C for 4 – 8 days. After that, cover slips were removed and observed under high power objective. The morphological features of spore and aerial mycelium were recorded. Biochemical tests were performed including IMVIC, Nitrate reduction, Urease, Catalase, Starch hydrolysis and Caesin hydrolysis tests.

4. Identification of actinobacteria on the basis of 16s RNA

Identification of actinobacteria on the basis of 16s RNA different material, chemicals,

Equipment and Glasswares and gel documentation system (Alpha Imager-2200, Alpha Innotech Corporation, USA).

5. Extraction of genomic DNA

The genomic DNA of each sample was extracted and purified by AxyPrep Bacterial Genomic DNA Miniprep kit as directed by the manufacturer's manual instructions and its purity and quantification were estimated by Nanodrop.

Universal 16S rRNA PCR forward primer (27f: AGAGTTTGATCMTGGCTCAG) and reverse primer (1492r: TACGGYTACCTTGTTACGACTT) were used in the amplification of 16S rRNA genes. Its sequences after sequencing were pasted in the window at the online BLAST analysis tool which provided results in the form of maximum similarity of all available sequences in Gene Bank.

6. **Phylogenic analysis of actinomycetes**

The evolutionary relationship was analyzed on the basis of r-DNA sequences using the UPGMA method. The percentage of replicate trees in which associated data is clustered together in bootstraps (1000 replicates) was shown next to branches (Felsenstien *et al*., 1985).

III. RESULTS AND DISCUSSIONS

1. To study morphology, cultural and biochemical study of Actinomycetes

The sample collection was performed for a period of three years from January 2015 to December 2018. During the study period around **60** water samples were collected. The primary analysis of the water samples including the colour, temperature and pH of the water was noted at the sample site. Further physic-chemical analysis of the water samples including the study of total dissolved solids (TDS), chloride, and salinity was conducted at the P.G. Department of Microbiology, Shri Shivaji Science and Arts College, Chikhli, district Buldhana. The results of the preliminary analysis of the samples have been mentioned in the table (**Table No. 1)** below-

Table 1: Preliminary physicochemical analysis of water samples from Lonar Lake of Buldhana District (M.S.).

Sr. No.	Sample spot	Season	Color	Temperature (^{0}C)	pH	TDS (mg/L)	Chloride (mg/L)	Salinity (mg/L)
1	A	Post Monsoon	LGY	27	10.3	9293	2993.53	5406.34
2	B		LGY	27	10.3			
3	C		LGY	27	10.3			
4	D		LGY	27	10.3			
5	E		LGY	27	10.3			
6	A	Summer	LGY	27	10.4	12380	3816.46	6892.56
7	B		LGY	27	10.4			
8	C		LGY	27	10.4			
9	D		LGY	27	10.4			
10	E		LGY	27	10.4			
11	A	Monsoon	LGY	27	10.2	8220	2803.21	5062.62
12	B		LGY	27	10.2			
13	C		LGY	27	10.2			
14	D		LGY	27	10.2			
15	E		LGY	27	10.2			

Where: A – Dhar Spot, B – Ramgaya spot, C – Kamalaji Devi temple spot, D – Ambarkhana Mahadev Temple spot, E – Mungala Mahadev Temple spot, LGY – Light Green color with yellow shade, TDS – Total Dissolved Solids.

2. Isolation, Identification and Characterization of Actinomycete Isolates

The isolation of actinomycetes was performed on two different mediums including Starch Casein Agar (SCA) and Actinomycete Isolation Agar (AIA). The samples were inoculated using the spread plate method and further incubated at 30^{0}C for 21 days and monitored every 24 hrs. After the observation of colonies with typical actinomycete morphology, the colonies were picked and further transferred to respective media plates for further study. During the study out **of 60 water samples 04** actinomycete isolates were obtained which were labelled from ACM1 to ACM 4.

Table 2: Isolates of Actinobacteria from Lonar Lake water:

S. No.	Actinobacteria Isolates
1	*Kocuria* sp.
2	*Conexibacter* sp.
3	*Brevibacterium* sp.
4	*Rubrobacter* sp.

3. Morphological characteristics of isolates of Actinobacteria from Lonar Lake water

- ***Kocuria* spp**.

  Dark black in colour, with circular, leathery, and non-fragmented vegetative mycelium, smooth walled spore aerial hyphae were black in colour.

- ***Conexibacter* spp.**
  White or dull white in colour, with circular, raised leathery colonies, brown pigment on reverse side.
- ***Brevibacterium* spp**.
  Brown, circular, center raised leathery, aerial mycelium grey in colour.
- ***Rubrobacter* spp.**
  Reddish brown substrate mycelium raised leathery colonies, brown pigment on reverse side.

Table 3: Colony characteristics of Actinomycete Isolates

Isolate	Medium Used	Pigmentation	Colony Morphology	Spore formation	Spore arrangement	Tentative genera
ACM 1	AIA	Brown	Circular, white large	-	-	*Kocuria*
ACM 2	AIA	-	Circular, green colour, centre faint green and periphery dark green	-	-	*Conexibacter*
ACM 3	AIA	Brown	Circular Black	-	-	*Brevibacteriun*
ACM 4	AIA	Brown	Circular, white, large	-	-	*Rubrobacter*

4. Biochemical Study

The results of the biochemical tests of actinomycete isolates have in mentioned in the tables (**Table 4 and 5**):-

Table 4: Biochemical Characteristics of Actinomycete isolates

S. N.	Actinobacteria Isolates	Indole	Methyl Red	Voges - Proskauer	Citrate Utilization	Nitrate reduction	Urease	Catalase
1	*Kocuria rhizophila*	+	+	-	+	-	+	-
2	*Conexibacter woesei*	+	+	-	-	-	+	-
3	*Brevibacterium* spp	+	+	-	-	+	-	+
4	*Rubrobacter xylanophilus*	+	+	-	-	+	-	+

Table 5: Biochemical Characteristics of Actinomycete isolates

S. N.	Actinobacteria Isolates	Starch hydrolysis	Casein hydrolysis	Sugar Fermentation		
				Glucose	Lactose	Mannitol
1	*Kocuria rhizophila*	-	+	A	A	-
2	*Conexibacter woesei*	+	+	A	A	-
3	*Brevibacterium* spp	-	-	A	A	-
4	*Rubrobacter xylanophilus*	-	-	A	A	-

5. Molecular identification and Phylogenetic analysis

Molecular identification was carried out on the basis of 16S rRNA gene sequences (Abdelmohsen *et al.,* 2014). The genomic DNA of 4 Actinomycetes isolates was extracted by using a commercially available kit according to the manufacturer's guide. The purity and DNA concentration were determined using Nanodrop. So, DNA obtained was pure and with a high concentration.

We have received accession numbers for all the sequences. The isolated actinomycetes species were ACM 01 *Kocuria rhizophila* (ON248525), ACM 02 *Conexibacter woesei* (ON377276), ACM 03 *Brevibacterium spp*., ACM 04 *Rubrobacter xylanophilus* (ON387757),

16s RNA sequences :-

ACM1 ***Kocuria rhizophila***

GTAGCCGACCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCACA
ATGGGCGAAAGCCTGATGGAGCAACGCCG
CGTGAGTGAAGAAGGATTTCGGTTCGTAAA
ACTCTGTTGTAAGGGAAGAACAAGTACAGT
AGTAACTGGCTGTACCTTGACGGTACCTTA
TTAGAAAGCCACGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGTGGCAAGCGTT
GTCCGGAATTATTGGGCGTAAAGCGCGCGC
AGGTGGTTTCTTAAGTCTGATGTGAAAGCC
CACGGCTCAACCGTGGAGGGTCATTGGAAA
CTGGGAGACTTGAGTGCAGAAGAGGATAG
TGGAATTCCAAGTGTAGCGGTGAAATGCGT
AGAGATTTGGAGGAACACCAGTGGCGAAG
GCGACTATCTGGTCTGTAACTGACACTGAG
GCGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGA
TGAGTGCTAAGTGTTAGGGGGTTTCCGCCC
CTTAGTGCTGCAGCTAACGCATTAAGCACT
CCGCCTGGGGAGTACGGTCGCAAGACTGA
AACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAG
CAACGCGAAGAACCTTACCAGGTCTTGACA
TCCCGTTGACCACTGTAGAGATATGGTTTC
CCCTTCGGGGGCAACGGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTGATCTTAGTTGCCATCATTTAGTTGGGCA
CTCTAAGGTGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAATCATCAT
GCCCCTTATGACCTGGGCTACACACGTGCT
ACAATGGACGATACAAACGGTTGCCAACTC
GCGAGAGGGAGCTAATCCGATAAAGTCGTT
CTCAGTTCGGATTGTAGGCTGCAA.

ACM 02 ***Conexibacter woesei***

GGGTAGCTGGTCTGAGAGGACGATCAGCC
ACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTAGGGAATCTTGCG
CAATGCGCGAAAGCGTGACGCAGCAACGC
CGCGTGGAGGAAGACGGTCTTCGGATTGTA
AACTCCTTTCAGTTGGGACGAAGCTTCGGC
GATGAATAGTCGTTCGGAGTGACGGTACCT
TCAGAAGAAGCCCCGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGGGGCGAGC
GTTGTCCGGAATCATTGGGCGTAAAGCGCG
TGTAGGCGGCTCGATAAGTCTGCTGTGAAA
GTCCAGGGCTCAACCCTGGAATGCCGGTGG
ATACTGTCGAGCTTGAGTCCGGAAGAGGCG
AGTGGAATTCCTGGTGTAGCGGTGAAATGC
GCAGATATCAGGAGGAACACCAATGGCGA
AGGCAGCTCGCTGGGACGTGACTGACGCTG
AGACGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCTGTAAAC
GATGGGTACTAGGTGTGGGGGGTGTCGACT
CCCTCCGTGCCGACGCTAACGCATTAAGTA
CCCCGCCTGGGGAGTACGGCCGCAAGGCTA
AAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCAGCGGAGCATGTGGTTTAATTCGAC
GCAACGCGAAGAACCTTACCTGGGCTTGAC
ATGTGCCTGACCGCCGTGGAAACACGGCTT
CCCTTCGGGGCAGGTTCACAGGTGGTGCAT
GGCTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCCC
GTCGCATGTTGCCAGCATTTAGTTGGGGAC
TCATGCGAGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAGTCATCAT
GCCCCTTATGTCCAGGGCTACACACGTGCT
ACATTGGCGCGTACAGAGGGCTGCGATACC
GCGAGGTGGAGCGAATCCCTTAAAGCGCGT
CTCGGTTCGGATTGTAGGCTGAAACTCGCC
TACATGAAGGCGGAGTTGCTAGTAATCGCG
GATCAGCATTGCCGCGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCAC
GGGAGTGAGCAACACCCGAAGCCGGTGGC
CTAACCCGCAAGGG

ACM 03 ***Brevibacterium spp.***

CGGGTGAGTAACACGTGGGCAACCTGCCTA
TAAGACTGGGATAACTTCGGGAAACCGGA
GCTAATACCGGATACGTTCTTTTCTCGCATG
AGAGAAGATGGAAAGACGGTTTACGCTGT
CACTTATAGATGGGCCCGCGGCGCATTAGC
TAGTTGGTGAGGTAATGGCTCACCAAGGCG
ACGATGCGTAGCCGACCTGAGAGGGTGATC
GGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCGCAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAACGAAGAAGGCCTTCGGG
TCGTAAAGTTCTGTTGTTAGGGAAGAACAA
GTACCAGAGTAACTGCTGGTACCTTGACGG
TACCTAACCAGAAAGCCACGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGTGGC

AAGCGTTGTCCGGAATTATTGGGCGTAAAG
CGCGCGCAGG
TGGTTCCTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTG
GGGAACTTGAGTGCAGAAGAGGAAAGTGG
AATTCCAAGTGTAGCGGTGAAATGCGTAGA
GATTTGGAGGAACACCAGTGGCGAAGGCG
ACTTTCTGGTCTGTAACTGACACTGAGGCG
CGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGA
GTGCTAAGTGTTAGAGGGTTTCCGCCCTTT
AGTGCTGCAGCTAACGCATTAAGCACTCCG
CCTGGGGAGTACGGCCGCAAGGCTGAAAC
TCAAAGGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAA
CGCGAAGAACCTTACCAGGTCTTGACATCC
TCTGACAACCCTAGAGATAGGGCTTTCCCC
TTCGGGGGACAGAGTGACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTT
GATCTTAGTTGCCAGCATTCAGTTGGGCAC
TCTAAGGTGACTGCCGGTGACAAACCGGAG
GAAGGTGGGGATGACGTCAAATCATCATGC
CCCTTATGACCTGGGCTACACACGTGCTAC
AATGGATGGTACAAAGGGCTGCAAACCTG
CGAAGGTAAGCGAATCCCATAAAGCCATTC
TCAGTTCGGATTGCAGGCTGCAACTCGCCT
GCATGAAGCCGGAATCGCTAGTAATCGCGG
ATCAGCATGCCGCGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACC

ACM 04 ***Rubrobacter xylanophilus***

GTCAGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGCCAGCAGCCAGGAA
TCTTGCGCAATGGGCGAAAGCCTGACGCAG
CAACGCCGCGTGGGCGATGAAGGCCTTCGG
GTCGTAAAGCCCTGTTGTCGGGGACGAAGG
GTGAAGGGTTAATAGCCCCTAGCCTGACGG
TACCCGACGAGGAAGCCCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGGG
CGAGCGTTGTCCGGAATCATTGGGCGTAAA
GAGCGTGTAGGCGGCCCGGTAAGTCTGCTG
TGAAAACCCGGGGCTCAACCCCGGGCGTGC
AGTGGAAACTGCCGGGCTAGAGGGCGGCA
GAGGCGAGTGGAATTCCCGGTGTAGCGGTG
AAATGCGCAGATATCGGGAGGAACACCAG
TAGCGAAGGCGGCTCGCTGGGCCGTTCCTG
ACGCTGAGACGCGAAAGCTAGGGGAGCGA
ACAGGATTAGATACCCTGGTAGTCCTAGCC
GTAAACGATGGGCACTAGGTGTGGGGGGA
GTCGAATCCCTCCGTGCCGCAGCCAACGCG
ATAAGTGCCCCGCCTGGGGAGTACGGCCGC
AAGGCTAAAACTCAAAAGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTTCTTTA
ATTCGATGCAACGCGAAGAACCTTACCTGG
GCTTGACATGCTGGTGGTAGGCGCCGGAAA
CGGTGTCCGACCCTTCGGGGAGCCAGCACA
GGTGCTGCATGGCTGTCGTCAGCTGTGTCG
TGAGATGTTGGGTTAAGTCCCACAACGAGC
GCAACCCCCGCCCTGTGTTGCCAGCATTAA
GTTGGGGACTCACAGGGGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTATGCCCAGGGCTAGA
AACGTGCTACAATGGCCGGTACAATGGGCT
GCGATGCCGCGAGGCGGAGCGAATCCCAT
CAAAGCCGGTCTAAGTTCGGATTGGAGTCT
GCAACTCGACTCCATGAAGGCGGAGTTGCT
AGTAATCGCGGATCAGCATGCCGCGGTGAA
TACGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCAC.

Table 6: Nucleotide sequence of ITS region of Actinomycetes

Sr. No.	Isolate No.	Actinomycetes Species	Gene Bank Accessions No.	% Similarity
1	ACM 01	*Kocuria rhizophila*	ON248525	100
2	ACM 02	*Conexibacter woesei*	ON377276	100
3	ACM 03	*Brevibacterium spp.*		100
4	ACM 04	*Rubrobacter xylanophilus*	ON387757	100

IV. DISCUSSIONS

Actinobacteria are known for their extraordinary potential to produce antibiotics. The present study is a small step towards the study of marine actinomycetes through the Lonar crater as the chief study site. Borul (2012) performed an analysis of the physicochemical properties of the Lonar lake water to analyse the quality of water of the lake. The authors focused on parameters like colour, odor, pH, temperature, TDS, chloride, magnesium, sulphate, phosphate and salinity of the water. The authors reported that the colour of the water was greenish with an offensive odour and a pH of 9.20 to 10.5 and a temperature 24°C to 27°C. The TDS of the water was in range of 14260 mg/L to 18460 mg/L and alkalinity was 3500mg/L to 4870mg/L. The total hardness was found to be 295 mg/L to 698 mg/L, chloride 4320 mg/L to 5650 mg/L, salinity 8460 mg/L to 10250 mg/L, sulphate 20 mg/L to 26.4 mg/L and phosphate 0.42 mg/L to 0.82 mg/L.

Sheik *et al.,* (2017) studied the morphological and biochemical identification of Actinobacteria from the soil sample of Ad-Dawadmi, Saudi Arabia and reported the isolation of 9 actinomycete isolates.

Monciardini *et al.,* (2002) developed selective primer sets for PCR amplification of 16S rDNA from the *Actinomycetales* families *Micromonosporaceae, Streptomycetaceae, Streptosporangiaceae and Thermomonosporaceae*, and from the genus *Dactylosporangium.* Each primer set, evaluated on genomic DNA from reference strains, showed high specificity and good sensitivity (Monciardini *et al.,* 2002). The application of these primers to environmental samples showed the frequent occurrence of these groups of actinomycetes and also revealed sequences that can be attributed to new groups of actinomycetes (Monciardini *et al.,* 2002). The results of Phylogenetic analysis of cloned 16S rRNA genes from Lonar lake (Wani *et al.,* 2006).

V. CONCLUSION

The search for novel bioactive secondary metabolites derived from microbial sources and which can be used as antibiotic compounds for their promising applications in the field of the agricultural, pharmaceutical, industrial and bio-medical fields has gained impetus due to the rising number of multi-drug-resistant pathogenic micro-organisms. Researchers throughout the world are consistently in search of novel, potent, broad-spectrum and sustainable antimicrobial compounds from several sources including microbes. As it is a fact that actinomycetes are prolific producers of bioactive compounds and *Streptomyces* species is one of the dominant species producing bioactive antibiotic compounds several researchers have focused on the screening and isolation of actinomycetes from a variety of ecosystems.

The present study also highlights the screening and isolation of actinomycete species from the Lonar crater, Buldhana district which has been a less unexplored area for isolation and research on marine actinomycetes. The results observed in the study reveal the isolation of 16 actinomycete isolates majorly belonging to *Streptomyces* species,

2 isolates of *Brevibacterium* species and single isolates of *Kocuria, Conexibacter,* Rubrobacter, *Actinomadura, Micromonospora, Nocardiopsis,* and *Actinobiospora*. The preliminary identification of the isolates was performed through morphological and biochemical methods while the confirmation of the genera and species was done using the 16S RNA and phylogenetic analysis. The antimicrobial study performed against the human pathogens micro-organisms also confirmed the antibiotic potential of the isolates.

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