



MICROBIAL DIVERSITY ANALYSIS OF DAL LAKE, INDIA USING 16S RRNA GENE BASED CULTURING APPROACH.

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ABSTRACT

Dal lake ecosystem (Lat. 340-6' N, 740-45'E, alt.1583) situated in the heart of Srinagar city the summer capital of Jammu and Kashmir state is under tremendous anthropogenic pressure for the last three decades. More than 50,000 people are living inside this hamlet, besides some people residing inside houseboats. People use this lake for their own purposes as this lake acts as livelihood for many families since centuries. Little work had been carried on Dal lake water and sediment samples to show the type of Microbial Diversity existing at present. By our work we tried to show whether anthropogenic activity is responsible for producing harmful bacteria. The traditional method of Microbial diversity analysis, culture dependent identification of species through morphological and biochemical test has not given the broad range of organism present in Dal Lake. Till date, only *Escherichia coli*, *Salmonella typhimurium* and *Penicillium sp.* were only identified. We have utilized 16S rRNA approach first time to study Microbial diversity in detail by taking water and sediment samples and had grown them on different media like Nutrient agar, Blood agar, and Eosine Methylene blue agar. As studies of 16S rRNA provides a microbial taxa present in a given sample because it is an excellent phylogenetic marker. Bacterial 16S rRNA sequences show the distribution of microorganisms predominantly in *Bacillus*, *Acinetobacter* and *Pseudomonas* species. As by our findings sediment samples show a variety of bacteria, whereas water samples showed presence of very few types of bacteria. The other bacteria recovered from the sediment samples include bacteria belonging to *Sporosarcina*, *Streptomyces*, *Arthrobacter*, *Rhodococcus*, *Enterobacter*, *Hydrogenophaga*, *Rheinheimeria*, and *Gammaproteobacterium* species. The other isolate recovered from the water samples belong to the species *Comamonas*, *Micrococcus*, *Rheinheimeria*, *Acidovorax*, *Sporosarcina*, *Paenisporosarcina*, *Rhodococcus*, *Bosea*, *Arthrobacter*. Based on literature survey, we can utilise the services of these bacteria for various purposes as these microorganisms can bring bioremediation activity in Dal lake such as degradation of crude oil, aromatic and alicyclic compounds, polynuclear aromatic hydrocarbons, mineral oils, synthetic polymers, styrene, heavy metals.

KEYWORDS :

Introduction:

The study of Microbial diversity is a basically characterization of microbial communities in nature. In the Microbial diversity analysis, one needed to discover, cultivate, isolate and characterize diverse microorganisms. It is estimated that ~99.9% of the microbes available in nature are not cultivable by using orthodox culturing techniques available. As a consequence of this, the vast majority of microbes which could be used in the agriculture, pharmacology and industry are out of human reach. Thus, researchers are interested to know the importance of their biotechnological as well as phylogenetic and ecological significance. Because of their dominance of global biogeochemical cycles, microorganisms are essential to life and the functioning of the biosphere (Fonknechten et al. 2008). However, the ecological importance of microorganisms has historically been overlooked. Today, this notion is replaced with a growing appreciation for their paramount importance and biodiversity (Curtis and Sloan, 2005).

Microbiology is the study of microscopic size organism which includes bacteria, fungi, algae, protozoa, and viruses. It also includes their distribution in nature, relationship with each other and other living organisms, their effects on human beings and other animals and plants. There is no field of human endeavor, where the microbe does not play an important and dominant role. The only aspect of their myriad actions that gets highlighted is their potential to cause misery, disease and injury. On the other hand, microbes are involved in the making of curd, cheese, butter, and wine, in the production of antibiotics like penicillin, manufacture of organic acids, alcohols and processing of domestic and industrial wastes. Microbes also play important role in most geochemical cycles, the world's climate (uptake of CO₂ in the Ocean), agriculture, human health, sustainable cities, reservoir for new drugs and metabolic processes (Griffith 1983). This enormous potential of different microbes is largely due to the degree of variation of life which is called Microbial diversity. Biodiversity on Earth is composed of three domains of life (Woese CR 1977). The three domains of life, Bacteria, Archaea and Eukaryotes, dominate life on our planet, as well as global biomass and carbon turnover (Whitman et al. 1998). Microbes are a large and diverse group of microscopic organisms that can live as single cells or in cell clusters. All bacteria and archaea are microbes, but domain Eukarya contains microbes as well as Fungi, Protozoa and microscopic Algae (Torsvik & Øvreås, 2011). The

domains, Archaea and Bacteria, are currently divided into several lineages, which constitute heterogeneous groups of species (Delong, 2003).

Materials and Methods:

For Microbial Community Analysis cultured approach 16S rRNA gene based approach was used. Sample collection: samples were collected using sterile plastic bottles, and stored for few days and later used for further analysis. Each sample were labeled as follows prior culturing them into different microbial media for growth.

Sample A: Bodal sediment 2,
Sample B: Gagribal sediment 1,
Sample C: Bodal Basin-Water sample 2,
Sample D: Gagribal basin-Water sample 1,
Sample E: Mixture of A B C D

Cultured Microbial diversity of Dal lake

Samples were spread on Nutrient Agar, Blood Agar and EMB Agar for growth of microorganisms. The sample was either serially diluted or directly spread on plate for growth of microorganisms. The scheme of spread plate has been detailed in the tables below. Total plate count for each culture was performed by observing the plates after 24 and 48 hours of incubation at 37°C. Plates were photographed and number of colonies were counted from each plate, the results were recorded.

Total viable count (TVC): For the determination of the total microbial count of culturable microorganisms in the different samples were grown in different growth media.

- **Nutrient Agar (NA):** The 0.1 ml inoculum was evenly spread over the entire surface of one of the nutrient agar plates until the medium no longer appears moist. Plates were incubated for 24-48 hours at 37°C and bacteria were enumerated.
- **Blood Agar (BA):** The 0.1 ml inoculum was evenly spread over the entire surface of one of the blood agar plates until the medium no longer appears moist. Plates were incubated for 24-48 hours at 37°C and bacteria were enumerated.

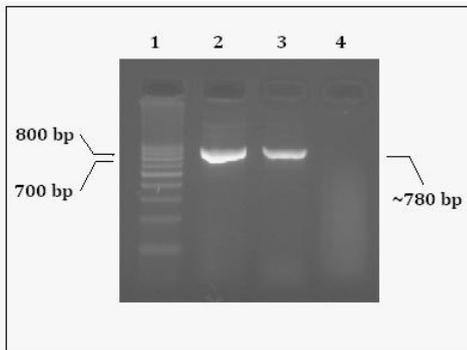
- **Ethylene Miosine Blue Agar (EMB):**The 0.1 ml inoculum was evenly spread over the entire surface of one of the EMB agar plates until the medium no longer appears moist. Plates were incubated for 24-48 hours at 37 °C and bacteria were enumerated.

Characteristics of colonies

Characteristics of colonies showing different morphological features as per visual observations were recorded.

Preparation of genomic DNA from pure cultures

Each single colony was picked up using sterile pipette tip and suspended in 100 µL sterile rapid microbial DNA preparation solution (0.1% Triton X-100 in Tris EDTA buffer; pH 8.2). The colony suspension was then boiled at 99 °C for 20 min in thermal cycler. The lysate after boiling was centrifuged at 10000 RPM for 10 minutes. 5µL of the supernatant was used as template DNA for 16S rRNA amplification.



Representative data for PCR amplification:

Agarose gel electrophoresis of 16S rRNA gene PCR products prepared from the culture sample of the Dal lake. Lane 1: 100bpLadder; Lane 2: PCR amplicons from colony; Lane3: Positive control colony PCR amplicon; Lane4: Negative control

Polymerase chain reaction of 16S rRNA

Polymerase chain reaction based amplification of 16S rRNA gene was carried out using geneOmbio Microbial Identification kit. The kit comprises of a primer sets targeting 16S rRNA from bacteria. Amplicon of 780 bp is generated by PCR amplification of 16S rRNA gene of bacteria. The PCR amplification reaction mix of 50 µl contained bacterial DNA, 1 µl (5 units) Taq-DNA polymerase, 5 µl of 10X PCR buffer 1 µl of 10 mM dNTP mix and 2 µl of each primer (10 pM/ µl). Amplification was carried out in a GeneAmp PCR System (Applied Biosystems, USA). In all the reactions, sterile water was used in place of DNA as a negative control. The thermal cycling program was: 94 °C for 2 minutes, 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute for 30 cycles and final extension of 72 °C for 10 minutes. The amplified DNA fragments of 780bp separated on a 2% agarose gel and purified by using geneOmbio PCR purification Kit (geneOmbio Technologies, Pune).

BLAST analysis 16S rRNA sequences were analyzed using BLAST analysis at National Centre for Biological Information (NCBI) online tool located at [www.HYPERLINK\"http://www.blast/\"http://www.blast.ncbi.nlm.nih.gov/](http://www.HYPERLINK\). The results of the analysis were recorded as identification of the organism.

Colony Forming Units Four accessible surface sites within the Dal lake were studied for the Total microbial count (Table 1). Using the culture-dependent technique of dilution plating, a wide range of CFU/ml values were observed for the samples. The Dal lake sites CFU data showed that for all media, the values range from 0 to 180×10⁴ CFUs/ml of sample. The bacterial number of sediment samples (Bodal and Gagribal basin) showed higher microbial load (CFU/ml) than the water samples (Bodal and Gagribal) on all the three principal media i. e. Nutrient agar, Blood agar and Eosin Methylene

blue. Of the 4 sites, water sample sites showed <1×10⁴ CFUs/ml while sediment samples showed 4×10⁴ CFUs/ml. Among sediment samples, Bodal sediment 2 sample represented higher microbial count on NA and BA then the Gagribal sediment 1 sample. Gagribal showed significantly higher number of CFU on EMB (Eosin Methylene Blue) agar plate which indicated the presence of a high number of Gram-negative bacteria in the sample. Water sample of Bodal basin showed 6500 CFU/ml on blood agar while a very less number of colonies were grown on NA(560 CFU/ml) and EMB(20 CFU/ml) plates. Gagribal basin showed 2000 CFU/ml microbial count when grown on nutrient agar plate while on BA and EMB agar plate only 830 and 80 CFU/ml, respectively. Mixture of sediment and water samples when plated for the microbial load count, with 55000 CFU/ml>37000 CFU/ml>18000 CFU/ml on NA, EMB and BA plate recorded, respectively.

Table 1. Total microbial count of water and sediment samples: Total microbial count (TVC), CFU/ml as determined by total viable count (TVC)

Sample	Plate Code	NA	BA	EMB
Bodal sediment 2	A	120×10 ⁴	180×10 ⁴	0.85×10 ⁴
Gagribal sediment 1	B	7.40×10 ⁴	9.0×10 ⁴	2.4×10 ⁴
Bodal Basin-Water sample 2	C	0.056×10 ⁴	0.65×10 ⁴	0.002×10 ⁴
Gagribal basin-Water sample 1	D	0.20×10 ⁴	0.083×10 ⁴	0.008×10 ⁴
Mixture of A B C D	E	5.50×10 ⁴	1.8×10 ⁴	3.7×10 ⁴

Morphological characterization of colonies The growing colonies were analyzed for various morphological parameters such as color, shape, size, margin, consistency, transparency and elevation. Most of the colonies were white in color, circular in shape, 1-3 mm in size with entire margin, moist consistency, opaque transparency and Umbonate/Raised/Flat or crateri form of elevation. The detailed morphological characterizations were presented in Table 2. Photographs of various plates with each sample were shown in plate pictures below

EMB agar Blood agar Nutrient agar Images plate A, Bodal Sediment 2



Images plate B, Gagribal sediment 1

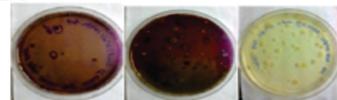


Image plate C, Bodal Basin-Water sample spread



Image plate D, Gagribal basin- Water sample 1



Image Plate E, Mixture of Sample A, B



Table 2. Morphological characterization of bacterial colonies isolated from Dal lake water samples.

S No.	Colony No.	Media	Colour	Shape	Size	Margin	Consistency	Transparency	Elevation
1	A1	BA	Whitish	Circular	1 mm	Entire	Moist	Translucent	Raised
2	A2	BA	Whitish	Circular	4 mm	Entire	Dry	Opaque	Umbonate
3	A3	BA	Whitish	Circular	1 mm	Entire	Moist	Opaque	Raised
4	A4	BA	Whitish	Circular	3 mm	Entire	Moist	Opaque	Umbonate
5	A5	EMB	Colourless	Circular	2 mm	Entire	Moist	Transparent	Raised
6	2A1	EMB	Pinkish	Circular	2 mm	Entire	Moist	Opaque	Flat
7	2A2	BA	Whitish	Circular	4 mm	Entire	Moist	Translucent	Crateriform
8	B1	BA	Whitish	Circular	3 mm	Entire	Dry	Opaque	Umbonate
9	B2	BA	Grey	Circular	5 mm	Entire	Moist	Opaque	Umbonate
10	B3	BA	Whitish	Circular	3 mm	Entire	Moist	Translucent	Flat
11	B4	BA	Grey	Irregular	4 mm	Undulate	Dry	Cloudy	Raised
12	B5	BA	Greenish	Rhizoid/Irregular	10 mm	Filiform	Dry	Cloudy	Convex
13	2B1	EMB	Whitish	Circular	1 mm	Entire	Moist	Opaque	Umbonate
14	2B2	EMB	Whitish	Circular	2 mm	Entire	Moist	Cloudy	Convex
15	C1	BA	Grey	Irregular	3 mm	Undulate	Moist	Opaque	Crateriform
16	C2	BA	Whitish	Circular	2 mm	Entire	Sticky	Transparent	Convex
17	C3	BA	Grey	Circular	4 mm	Rough	Moist	Opaque	Crateriform
18	C4	BA	Whitish	Circular	3 mm	Undulate	Moist	Opaque	Umbonate
19	C5	BA	Reddish	Circular	2 mm	Irregular	Moist	Opaque	Crateriform
20	2C1	EMB	Pinkish	Irregular	3 mm	Undulate	Dry	Translucent	Flat
21	2C2	EMB	Pinkish	Circular	3 mm	Undulate	Moist	Translucent	Flat
22	D1	BA	Grey	Circular	7 mm	Entire	Sticky	Opaque	Umbonate
23	D2	BA	Blackish	Circular	5 mm	Entire	Sticky	Translucent	Umbonate
24	D3	BA	Black	Circular	3 mm	Undulate	Dry	Opaque	Flat
25	D4	BA	Greenish	Circular	7 mm	Undulate	Dry	Opaque	Flat
26	D5	BA	Yellowish	Circular	2 mm	Entire	Moist	Opaque	Raised
27	2D1	EMB	Pinkish	Circular	1 mm	Entire	Moist	Iridescent	Raised
28	2D2	EMB	Pinkish	Circular	1 mm	Entire	Indecent	Raised	Raised

Table 3. Nearest neighbor of 16S rRNA isolate obtained from cultured diversity of Dal lake in summer season, 2011-12.

Sample ID	Plate Code	Culture Code	Blast Hit	Gen Bank Accession number	Max. identity
Bodal Sediment 2	A	A1	Bacillus subtilis BaAP3	JQ734770	97%
		A2	Bacillus cereus AIMST 3ME9S	JQ311956	88%
		A3	Bacillus megaterium BVC2	JQ660585	91%
		A4	Bacillus megaterium Bacteria_179	JQ800443	96%
		A5	Thalassobacillus devorans AS11	HE586575	80%
		2A1	Acinetobacter tandoii CCM 7199	HQ180189	78%
		2A2	Leclercia adecarboxylata AIMST	Ehe6 JQ312039	91%
Gagrival Sediment A	B	B1	Bacillus thuringiensis parans KU23	JF895487	96%
		B2	Bacillus megaterium WIF19	HM480313	91%
		B4	Bacillus nanhaiensis C3PO3	JQ689197	93%
		2B1	Paenibacillus scineris BAC1091	HM355680	89%
		2B2	Acinetobacter sp. JB54	EF103571	88%
Bodal Basin Water Sample 2	C	C1	Bacillus pumilus VIT BP	FJ743437	91%
		C2	Pseudomonas sp. HKF-3	AB633201	97%
		C3	Bacillus aryabhatai NIOF	JQ818352	90%
		C4	Pseudomonas sp. D65lp(2011)	JN228318	87%
		C5	Bacillus pumilus M5	JX312616	97%
		2C1	Pseudomonas anguillisept	DQ518917	87%
		2C2	Pseudomonas anguilliseptica, E141	FM991861	91%

Gagribal Basin Water Sample 1	D3	Bacilluspumilus,	AM778180	85%
	D4	Bacilluspumilus S7	GU969592	96%
	2D1	Acinetobacter sp. SF119	AM490040	87%
	2D2	Acinetobacter sp. EU60	JF681287	88%

Table 4. Nearest neighbor of 16S Rrna isolates obtained from cultured diversity of Dal lake in winter season, 2011-2012.

Sample ID	Plate Code	Culture Code	Closest Blast Hit	Percent Similarity
Bodal Sediment 2	A	A1	Acinetobacter sp. sw-6-1(2011)	99%
		A2	Bacillus sp. BMR2	94%
		A3	Sporosarcina sp. Eur1 9. 8	95%
		A4	Streptomyces sp. FXJ7. 104	92%
		A5	Rhodococcusmaanshanensis GMC121	89%
		A6	Bacillus sp. NOB11	97%
		A7	Bacillusaquimaris PL29	73%
		A8	Streptomyces sp. 14CM003	93%
		A9	Acinetobacter sp. 4 JDE-2009, isolate 4	94%
		A10	Acinetobacter lwoffii G26	97%
		A11	Sporosarcina sp. TmT2-26	95%
		A12	Arthrobacteroxydans WA1-10	99%
		A13	Pseudomonasfluorescens NBRC 15830	99%
		A14	Enterobacter cloacae C111	94%
		A15	Enterobacter cloacae Bi37	93%
Gagribal Sediment 1	B	B1	Pseudomonassp. D65lp	97%
		B2	Pseudomonassp. HKF-3	99%
		B3	Hydrogenophaga pseudoflava KS6	89%
		B4	Hydrogenophaga taeniospiralis C2PO1	83%
		B5	Gamma proteobacterium GPTSA100-22	80%
		B6	Rheinheimera taxanensis TSWCW2	85%
		B7	Pseudomonas anguilliseptica K1946	71%

	B8	Brachybacterium sp. BA-142	94%
	B9	Pseudomonassp. D65lp(2011)	99%
	B10	Phenylobacterium sp. I_10-G7401D6	89%
	B11	Rheinheimera sp. C3-4m	88%
	B12	Rheinheimera sp. C3-4m	97%
	B13	Hydrogenophaga sp. p3(2011)	99%
	B14	Bacilluscereus NIOAD14	88%
	B15	Pseudomonassp. BCBo5, EU140959	88%

Table 5. Bioremediation features of cultured microorganisms obtained from Dal lake.

Name of Organism	Phylum	Habitat	Properties	References
Acinetobacter sp.	Proteobacteria	Commonly occur in soil, water, or wastewater	Degrade recalcitrant aromatic and alicyclic compounds, as well as some aromatic amino acids, mineral oils, and synthetic polymers; emediation of Atrazine, polynuclear aromatic hydrocarbons, Cr(VI)	Hendrickx et al. 2003,Wang ZG et al. 2011, JOSHI & LEE. 1996, Panda and Sarkar, 2012.
Sporosarcina sp.	Firmicutes	Soil of Urumqi, China	Remediation of As(III)	Varenyam et al. 2012
Streptomyces sp.	Actinobacteria	Loktak lake, Manipur, India; soil ofprotected forest areas from the states of Assam and Tripura, India	Reduce Cr(VI), Lindane	Polti et al. 2010, Singh et al. 2006, Thakur et al. 2007, Salam and Das. 2012
Rhodococcus maanshanensis	Actinobacteria	Maanshan Mountain, Anhui Province, China Maanshan	Nitrate, adenine, aesculin, arbutin and Tweens 20, 60 and 80	Zhang et al. 2002
Bacillus aquimaris	Firmicutes	Tidal flat of the Yellow Sea in Korea; Yangtze River, China	Heavy metals	Zhang et al. 2014, Yoon et al. 2003

Acinetobacter lwoffii	Proteobacteria	Andean Lakes (HAAL) of the Aniline South American Andes	Aniline degradation, Cr(VI) reduction South American Andes Phenol-degrading	Kim et al. 2001,Guosheng et al. 2011, Albarracin et al. 2012
Arthrobacter oxydans	Actinobacteria	Freshwater lake in Antarctica	Trivalent and Hexavalent chromium, organophosphate insecticide diazinon, degrading benzo[a]pyrene, nitrate reduction	Peng et al. 2012, Loveland-Curtze et al. 1999
Pseudomonas fluorescens	Proteobacteria	Proteobacteria Eastern and southeaster Sicily (Italy)	Degrading styrene, TNT, polycyclic aromatic hydrocarbons; antiphytopathogenic and biocontrol properties, produces phloroglucinol, phloroglucinol carboxylic acid and diacetylphloroglucinol	Noura et al. 2009
Enterobacter cloacae	Proteobacteria	Lake DeForest, New City, New York	Se(VI) Reduction and the precipitation of Se(0)	Yee et al. 2007
Pseudomonas sp.	Proteobacteria	commonly occur in soil and water	Degrade polycyclic aromatic hydrocarbons, reduced Cu and Ni	Kumar et al. 2010
Hydrogenophaga pseudoflava	Proteobacteria	Heywood Lake, Signy Island, Antarctica; Weende, Federalarea, USSR Republic of Germany	Denitrification, hydrogen-oxidizing bacteria	Willems et al. 1989
Hydrogenophaga taeniospiralis	Proteobacteria	Heywood Lake, Signy Island, Antarctica; Water, River Weende, Federalarea, USSR Republic of Germany Western Ghats, India;	Denitrification, Reduction of nitrate and nitrite; hydrogen-oxidizing bacteria, degrade polychlorinated biphenyls	Willems et al. 1989, Lambo and Patel, 2006

Rheinheimera taxanensis	Proteobacteria	Spring Lake, San Marcos, Texas; Sulfidic water (Movile Cave, Romania)	Sulphur reduction	Bhattacharya and Chakrabarti, 2009; Merchant et al. 2007
Acinetobacter calcoaceticus	Proteobacteria	Human body normal flora	Crude oil degradation	Lal and Khanna, 1996
Pseudomonas stutzeri	Proteobacteria	Human spinal fluid Gottingen,	Denitrifying, mineralizes carbon tetrachloride, degrade Crude oil, oil derivatives, and aliphatic hydrocarbons, aromatic hydrocarbons, Biocides	Grüntzig et al. 2001, Sepulveda-Torres et al. 1999, Grimberg et al. 1996, Liao et al. 2010, Lalucat et al. 2006
Bacillus niacini	Firmicutes	Federal Republic of Germany; hot springs in Thailand	Nicotinate-metabolizing, nitrate Reduction	Nagel &Andresen, 1991; Pakpitcharoen et al. 2008; Clare et al. 2012
Paenisporosarcina macmurdoensis	Firmicutes	Pindari glacier McMurdo Dry Valleys, Antarctica	Bioremediation of crude oil	Krishnamurthi et al. 2009;Reddy et al. 2003
Rhodococcus equi	Actinobacteria	Kuwait desert soil	Hexadecane degradation,bioremediation of crude oil; degrade ethyl tert-butyl ether	Bouchez-Naitali et al. 2001, Cho et al. 1999 Fayolle et al. 1998 Samanta et al. 2002

Pseudomonas putida	Proteobacteria	Moist soil and water habitats activated sludge from an aerobic-anaerobic wastewater	Degrade Crude Petroleum including Naphthalene, phenanthrene and salicylate; Biodegradation of benzene, toluene, ethylbenzene, and o-xylene; Trichloroethylene Degradation, reduce chromate	Nwachukwu, 2001 Zylstra et al. 1989, Ackerley et al. 2004, Shim and Yang, 1999
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Acidovorax sp.	Proteobacteria	Treatment plant (Bourgoyen-Ossemereen) in Gent, Belgium Churince system at Cuatro Ciénegas Basin (CCB) in the Mexican State of Coahuila; Uranim Ore Deposit of Domiasiat, North East India	Denitrifying	Heylen et al. 2008.
Pseudomonas koreensis	Proteobacteria	Uranim Ore Deposit of Domiasiat, North East India	Produce a biosurfactant, degradation of the polyethylene	Toribio et al. 2011, Yoon et al. 2012, Kumaret al. 2013.
Serratia rubidaea	Proteobacteria	Clinical samples	Produce biosurfactants	Matsuyama et al. 1990
Bacillus subtilis	Firmicutes	Lonar Lake, India	Remediation of hydrocarbons; convert some explosives into harmless compounds of N, CO ₂ , and water; radionuclide waste [thorium and plutonium] disposal	Joshi et al. 2008
Bacillus cereus	Firmicutes	Puliket marine back water lake, Chennai, India, Lake kasumigaur	remediation of mercury, degradation of phenol, Chromium remediation	Santos-Gandelman et al. 2014, Gayathri et al. 2010, Nobuyuki et al. 2003.
Bacillus megaterium	Firmicutes	Tokwawan, Hong Kong SAR	Remediation of Nitrate, Cr ⁶⁺ biodegradation of crude oil	Cheung et al. 2005, Thavasi et al. 2011
Thalassobacillus devorans	Firmicutes	Hypersaline habitats of southern Spain	phenol-degradation	García et al. 2005
Bacillus thioparans	Firmicutes	Estero de Urias coastal lagoon near Mazatlán, Sinaloa, México	Cu and Pb biosorption, thiosulfate-oxidizing bacterium	Rodríguez-Tirado et al. 2012; Pérez-Ibarra et al. 2007
Bacillus nanhaiensis	Firmicutes	Naozhou Island, the South China Sea	Yet not known	Chen et al. 2011.

Paenibacillus cineris	Firmicutes	Antarctic volcanic soils	Nitrogen fixation	Logan et al. 2004
Bacillus pumilus	Firmicutes	Cochin, West coast of India	Production of antifungal compound and biosurfactant	Parvathi et al. 2009.
Bacillus aryabhatai	Firmicutes	East Kolkata, Wetlands	Zinc solubilizing abilities, Cr ³⁺ , removal of hexavalent chromium	Ray et al. 2012, Verma et al. 2014, Ramesha et al. 2014
Acinetobacter tandoii	Proteobacteria	Victoria, Australia	Avermectin biodegradation	Carr et al. 2003
Leclercia adecarboxylata	Proteobacteria	Digboi oil refinery, India; fallow land, botanical garden of the University of Ilorin, Nigeria.	Hydrocarbon-degrading capability	David et al. 2014
Pseudomonas anguilliseptica	Proteobacteria	Shizuoka Prefecture, Japan; filling stations in Biharkeresztés and Zalaegerszeg and, in Ópusztaszer, Hungari	Hydrocarbon-degradation	István S. 2011

Phylogenetic relationships of taxa

Evolutionary processes in large populations of bacteria are not well understood at present. Phylogenies are a fundamental tool for organizing biological diversity. A phylogenetic tree, also known as a phylogeny, is a diagram that depicts the lines of evolutionary descent of different species, organisms, or genes from a common ancestor (Xia X, Xie Z, 2001). The fine structure of phylogenetic trees can provide useful information about evolution and functional specialization in natural microbial populations. Phylogenies are useful for organizing knowledge of biological diversity, for structuring classifications, and for providing insight into events that occurred during evolution. Tree diagrams have been used in evolutionary biology since the time of Charles Darwin. Therefore, one might assume that, by now, most scientists would be exceedingly comfortable with "tree thinking"--reading and interpreting phylogenies (Yee N, Ma J, 2007).

As one of the aim of this study was to determine the biodiversity and community structure within sediments and water samples of Dal lake. Clone libraries were chosen as it provides useful data for further analysis of the Dal lake environment (Wayne LG 1987). The isolates in some phylogenetic group showed a similar distribution between water and sediment sample of Dal lake (Widdel F, 1982). The community structure in sample D was comparatively more complex than the sample A, B, C and E.

An isolate designated 2A2 branched deeply within Enterobacter, Pseudomonas and Acinetobacter genus, could not be assigned a single phylogenetic group and appears to represent a novel lineage. Phylogenetic analysis showed that A1, A2, A4 and A6 were related to

the remediation of hydrocarbons, radionuclide waste, Cr6+, chromium and nitrate, crude oil and degradation of phenol as shown in bioremediation table earlier. A5 was most similar to *Rhodococcus maanshanensis*(89% similarity) by BLAST search. A1, A3, A8, A12, A13 were most similar to *Bacillus pumilus*, *Sporosarcina sp.*, *Streptomyces sp.*, *Arthrobacter oxydans*, and *Pseudomonas fluorescens*, respectively. This relationship is also supported by a 100% bootstrap value in the phylogenetic tree. The selenium is an essential micronutrient, the higher valence states of Se(VI) and Se(IV) are toxic at elevated concentrations and can cause severe poisoning of fish and waterfowl in contaminated environments. *Enterobacter cloacae* cause reduction of soluble selenate [Se(VI), SeO4²⁻] toxic Se(0) converts selenium into an insoluble mineral form (Yee et al. 2007).

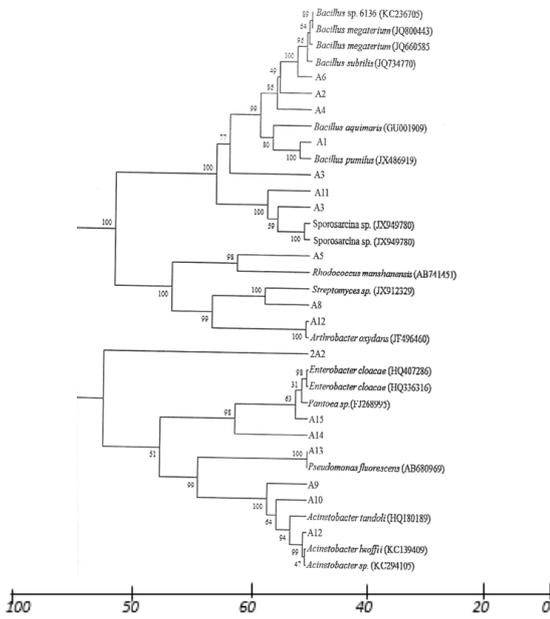


Figure 1. Phylogenetic tree of bacterial 16S rRNA half reaction of isolates derived from the Dal Lake Sample, Bodal sediment 2. The GenBank accession codes are given Bootstrap values at branch node also given.

Analysis of Sample B using larger data set which included many known bacterial divisions and candidate divisions suggested it was distantly allied with the Hydrogenophaga taeniospira division (Figure 2)

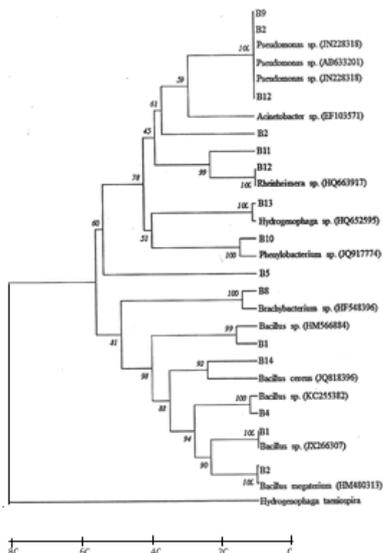


Figure 2: Phylogenetic tree of bacterial 16S rRNA clones derived from the Dal Lake cultured sample, Gagribal sediment1 The GenBank accession codes are given in parentheses. Bootstrap values are presented at branch node.

In Sample C, another group of phylotypes (C1, C2, C22, C42, C82, C92 and C112) forms another distinct deep branching cluster (Figure 3).

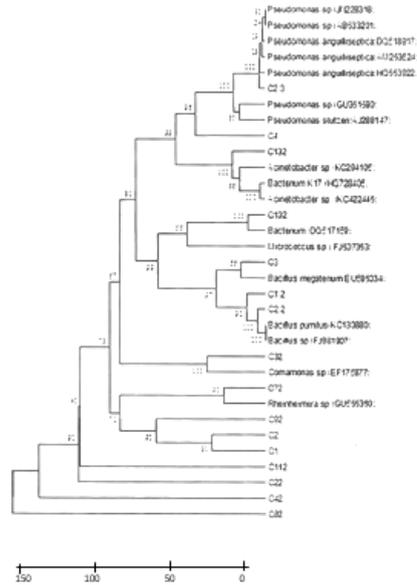


Figure 3. Phylogenetic tree of bacterial 16S rRNA clones derived from the Dal Lake cultured diversity Bodal Basin Water Sample. The GenBank accession codes were given in parentheses. Bootstrap values are presented at branch node.

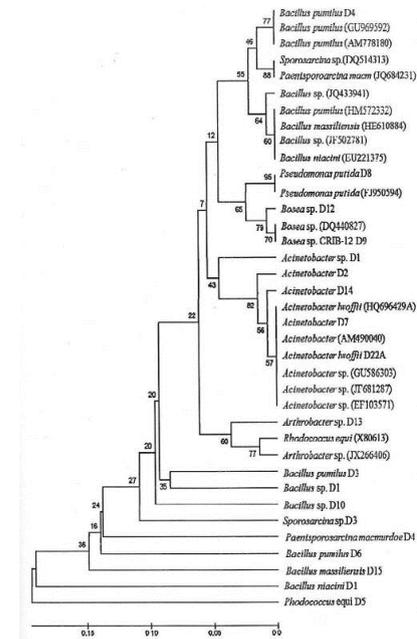


Figure 4. Phylogenetic tree of bacterial 16S rRNA clones derived from Dal Lake cultured Gagribal basin - Water sample . Bootstrap values are presented at Branch node.

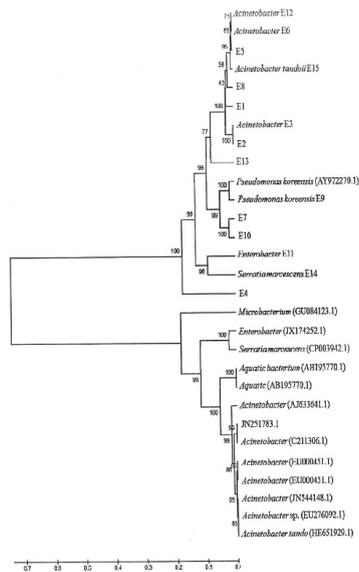


Figure 5. Phylogenetic tree of bacterial 16S rRNA clones derived from amixture of cultured samples from Dal Lake . The sample contains Bodal Sediment 2, Gagribal sediment , Bodal Basin –Water sample 2, Gagribal basin- water sample 1. The GenBank accession codes are given. Bootstrap values are given at branch node.

Pervious work done for Microbial diversity in Dal lake:

The literature survey showed very limited study carried out on Dal lake for the Microbial diversity analysis. The few reported methods have utilized culture dependent approach to describe microbial community in Dal lake. Saleem et al. (2011) has reported the most dominant bacterial species in Dal lake were *E. coli* (15.77%) > *E. aerogenes* (12.19%) > *Bacillus sp.* (11.96%) > *S. aureus* (10.85%) > *M. luteus* (10.17%) > *P. aeruginosa* (8.27%) > *K. pneumoniae* (6.71%) > *V. cholerae* (6.59%) > *Salmonella sp.* (6.15%) > *S. marcescens* (5.92%) > *C. freundii* (5.59%). They have also isolated total 213 fungal colonies, from which six species of *Penicillium* viz, *P. caseicola*, *P. commune*, *P. chrysogenum*, *P. funiculosum*, *P. lilacinum*,

Penicillium sp. and six species of *Aspergillus* viz, *A. flavus*, *A. fumigatus*, *A. japonicas*, *A. terreus*, *A. niger* and *Aspergillus spp.* Were selectively isolated. Out of these species, they found *P. chrysogenum* was the most abundant (30.99%) followed by *P. funiculosum* (16.43%) > *A. fumigatus* (14.09%) > *A. niger* (13.15%) > *A. flavus* (9.39%) > *A. terreus* (3.76%) > *P. lilacinum* (3.27%) > *P. caseicola* (2.82%) > *P. commune* (2.35%) > *A. japonicas* (1.88%) > *Penicillium sp.* And *Aspergillus sp.* (0.94%). In another study, a bacterium designated BzDSO3 was isolated from water sample and characterized by using 16S rRNA gene and 16S-23S rRNA internal transcribed spacer region sequences. Phylogenetic analysis showed 99% 16S rRNA gene sequence (Gene bank accession # FJ961336) similarity with *Escherichia coli* (Magray et al. 2011). However, this study has not described other possible species in the Dal lake. Thus it was clear that Dal lake needed to study at 16S rRNA level.

Conclusion

This research work first time highlighted the type of Microbial Diversity of Dal Lake. There was little work done in the past related to exploration of Microbial diversity but we used 16S rRNA approach for both sediment and water samples choosing almost hundred isolates. We further tried to explore how we can utilize their services for mankind. We found bacterial diversity which resembled Microbial diversity found all over the globe. And in the future we will try to explore the importance of those bacterial isolates which show least resemblance with the information present in the Gene

bank.

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Authors contribution

Conceived and designed the experiments, executed the experiments. Analysed the data. Reagents were used taking permission from Director and Co-guide from National Centre for Cell Science Pune, and some kits were used from Geneom Bio Pune.

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