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Bacterial Diversity Analysis of Upper Lake, Bhopal (M.P) India, Using Metagenomics

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ABSTRACT

Diversity analysis in reference to current methodology offers advantage over existing methodology such as biochemical characterization etc. Present study is carried out in order to establish diversity pattern using metagenomic procedure. The water body situated in Bhopal has its own recreation and water supply potential. Any water body is entertained by biological activity preferably predominant in sediment, as sediment represent site of various biological and geochemical activity. The protocols combine the application of mechanical (Beads and Sonicator) and soft lysis (SDS and enzyme) method for the isolation of total DNA from sediment of Upper lake of Bhopal followed by its quantification and purity assessment. The quality and purity of metagenomic DNA was quite consistent and reliable, although it contained residual concentration of humic acid. The methods have wide applicability in investigating molecular diversity and exploring functional genes.

The methodology used showed supportive observation of sediment habitat as use of extracted DNA when amplified using 16s rDNA region. Phylogenetic relationship was established. Dendogram was prepared using NTSYS (software for clustering of the data) which is based on unweighted Pair Group Method with Arithmetic Averaging (UPGMA). The dendogram showed deviation of initial two groups at 27% as well as 42% similarity. Sample B1 and B13

from Boat club site falls with absolute similarity, B4 and B10 also shows absolute resemblance in relation to pattern of amplification.

Key Words: NTSYS, DNA, rDNA

Introduction

Lakes accumulate sediments continually, a process that has been ongoing since their formation, in many cases for several thousand years or even longer. The sediment consists of biological remains from the lake itself and its surroundings, as well as soil particles and other non-biological material originating from the lake catchment and also the atmosphere. Hence, the sediment sequence in each lake is a continuous environmental archive, which contains information about the history of lake and its surroundings. The deposition rate of lake sediments is controlled by many factors. Lake morphology (area, depth, shoreline's length), water circulation, climate conditions, area and land use in the catchment, are only some of these features. A wide variety of climate-related information can be extracted and investigated from lake sediments. Some physical and geochemical properties of lake sediments can be related to climate variables. For example, the thickness of annual layers contains valuable information about past productivity or erosion input. The climate reconstruction based on non biological and biological remains that can be called as climate

'proxy' indicators(that is, they serve as an approximation or replacement for the missing instrumental data) are distinguishable. Like genomics itself, metagenomics is both a set of *research techniques*, comprising many related approaches and methods, and a *research field.* In Greek, *meta* means "transcendent" (beyond or above the range of normal or physical human experience). The other source of pollution includes sewage, comprising decomposable organic matter & pathogenic agent, agricultural pollutions and physical pollutants (Bhupendra et al. 2015)

The impact of molecular studies of microbial diversity cannot be overlooked. As a consequence, emphasis and focus on microbiology, from basic ecological research into the organization of microbial communities to bioprospecting for commercially relevant enzymes and metabolic potential, have changed (Lakay et al. 2007; Mitchell et al. 2008).

Metagenomic approaches also highlight on the population heterogeneity and phylogenetic status of a habitat in totality (Gabor et al. 2003; Galperin 2008; Mes 2008). Detecting the rare members of a microbial community is a challenge; however, it is very important as they play a critical ecological role (Yeates et al. 1998; Voget et al. 2003) in sediment ecosystem. The genetic information on all indigenous organisms can be accessed theoretically, including the predominant fraction of microorganism that is recalcitrant to cultivation, by applying metagenomics approaches (Raes et al. 2007). The biotechnological applications currently targeting microbial metagenomic studies range from the search for new antibiotics to environmentally sound biocatalysts (Mitchell et al. 2008).

In its approaches and methods, metagenomics circumvents the unculturability and genomic diversity of most microbes, the biggest roadblocks to advances in clinical and environmental microbiology.

Material & Method

1. Sample Collection : Sediment sample from Boat club, of Upper lake Bhopal was collected and brought to laboratory.
2. Metagenomic DNA was isolated using Bathe's method (Stephan Bathe, et. al., 2001) supernatant fractions were extracted with 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1), followed by 1 volume of chloroform/isoamyl alcohol (24:1), precipitated with 0.6 volume of isopropanol, washed with 70% ethanol and dissolved in 650 µl TE (6.5 ml). Crude DNA extracts were stored at -20^{o}C for further use.
3. 16s rDNA was amplified using forward and reverse primer (Table.2) in a thermo cycler.
4. RFLP analysis of 16s rDNA PCR Product; RAPD- PCR and Electrophoresis of PCR product. The electrophoresis techniques use for analysis of PCR product helped in separation DNA fragment according to molecular weight in the presence of agrose in 1x TAE buffer.
5. Cluster analysis: Genomic finger printing was examined using clustering method in terms of Dendrogram. The resulting bands were examined with the help of NTSYS for clustering of Data which is based upon unweighted pair group method with Arithmetic average (UGPMA)

Table.1 PCR cycle for amplification of 16S rDNA

Amplification stage	Temperature (^{0}C)	Time
Initial Denaturation	94	5min
Denaturation	94 (30cycle)	30 sec.
Annealing	50 (30cycle)	40sec.
Extension	72 (30cycle)	90sec
Final extension	72 (30cycle)	7 min.

Table 2 Primers used for amplification of 16s rDNA region.

S.No	Primer	Sequence
1.	Reverse	(5' AGAGTTTGATCCTGGCTCAG-3')
2.	Forward	(5'-AAGGAGGTGATCCAGCC GCA-3')

Table.3 Restriction Enzymes Used for RFLP

S.No.	**Enzyme**	**Sequence**	**Assays Temp.**
1.	**Alu I**	**AG↓C**	**37^{0}c**
2.	**Msp I**	**CC ↓GA**	**37^{0}c**
3.	**Hind III**	**A↓AGC**	**37^{0}c**
4.	**Taq I**	**T↓CGA**	**37^{0}c**

Table.4 Primers Used for RAPD analysis

S.No	Primer Name	Primer Sequence
1	Col 1	3-AAG AGC CCG T-5
2	GEN 15009	5-AGA AGC GAT G-3
3	OPC 9	3-CTC ACC GTC C-5
4	API 2	5-GTT TCG CTC C-3
5	E 2	5-GAA ATC TTT ATA CTT TCT TAA-3

Result and Discussion

During the study period samples were collected from Boat club from the Upper lake of Bhopal. Sediment of lake is predominant in microbial flora due to several biochemical activities, as many biological components gets deposited.

Bhopal is situated at equatorial plane minimum average temperature of upper lake recorded is 20.5^{0}C and higher site i.e. maximum temperature goes upto 32.5^{0}C. The availability of dissolved oxygen is regulated by temperature of the water body. At neutral

pH and 25^{0}C, most oxygenated lake water has redox potential of about +500mV (Fig. 1)

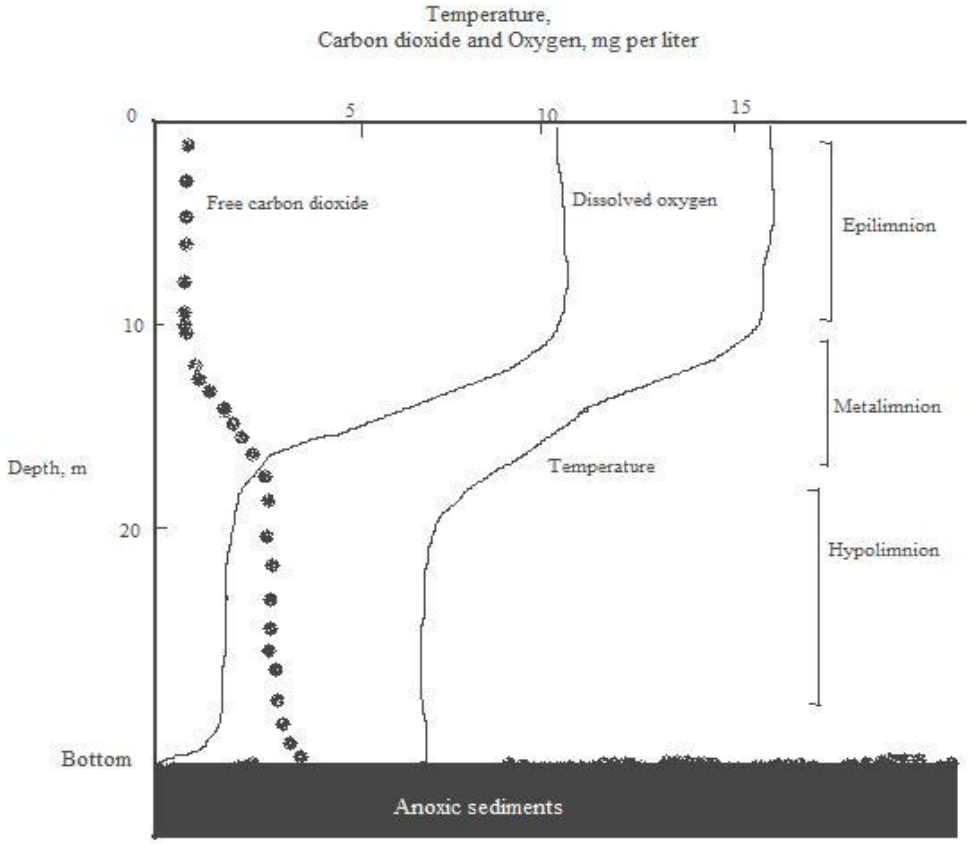

Fig.1 Diagrammatic representation of dissolved oxygen, CO_2 and temperature in summer in a productive lake.

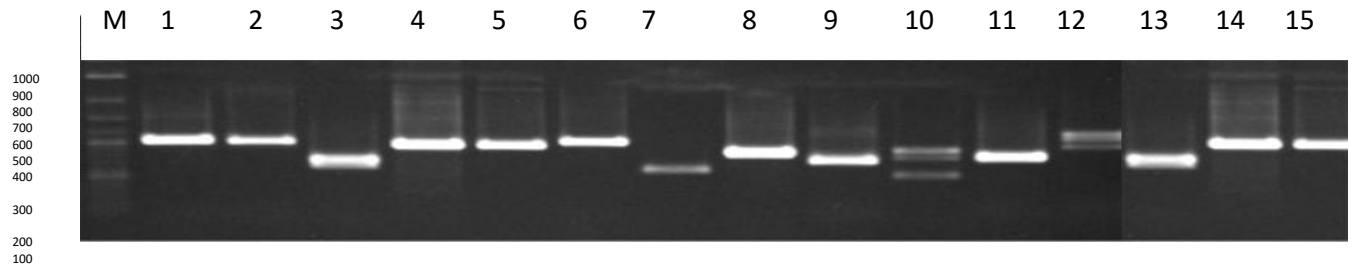

Fig.2 16Sr DNA PCR fingerprinting of DNA samples isolated from Boat club site of Upper Lake Bhopal on 2 % agarose gel with universal primers.

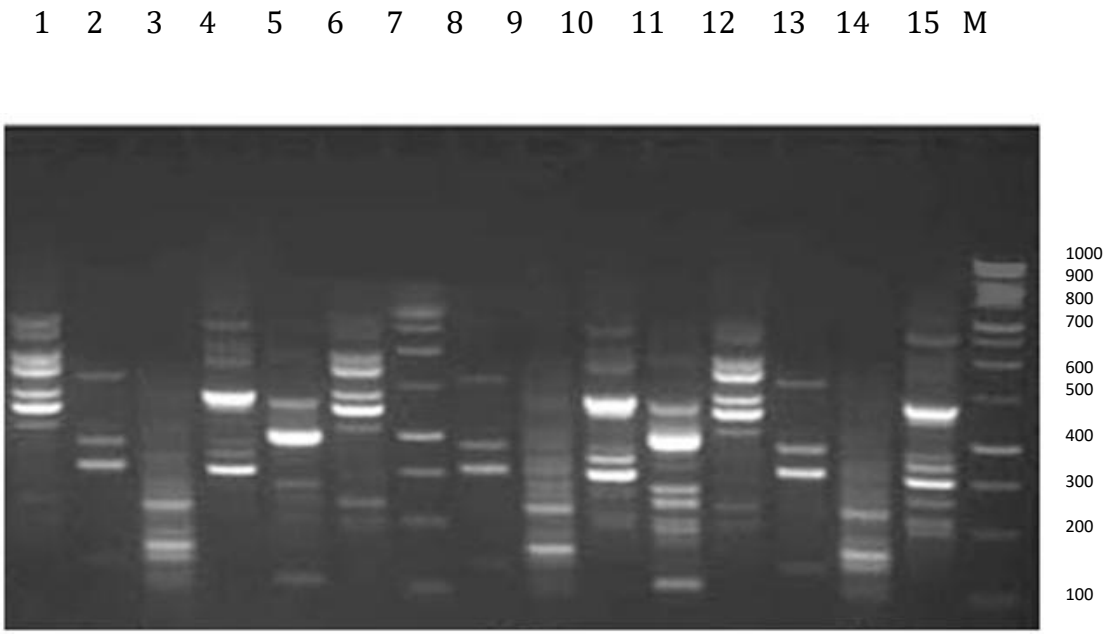

Fig.3 RAPD -PCR fingerprinting of DNA samples isolated from Boat club site of Upper Lake Bhopal on 2 % agarose gel with primer Col 1.

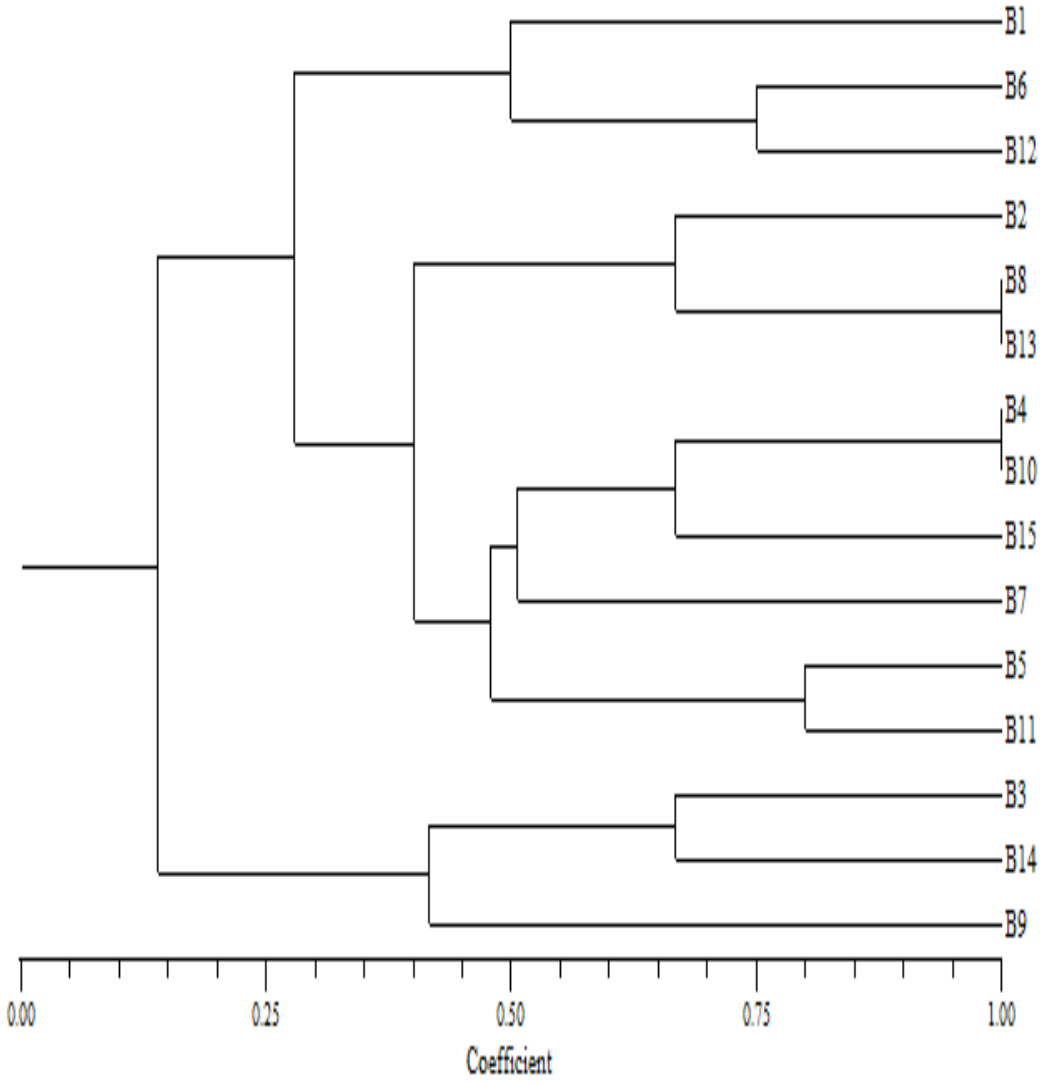

Fig.4 Dendrogram based on RAPD-PCR of DNA isolates from Boat club site of Upper Lake Bhopal showing genetic relatedness using Jaccard's similarity coefficient and UPGMA cluster method.

Microbes are deeply involved in all necessary reaction going on in biotic and abiotic scenario. Various methodologies developed recently are helpful in establishment of

presence of organisms and their genetic diversity. The diversity amongst organisms and their relatedness is determined by base sequence i.e., purines and pyrimidines present on their subsequent genetic material. The quantity of genome in relation to base density reflects good reasons of genetic discrepancy because expression is determined by the functional genome.

Irrespective of the physiological and biochemical properties samples from Boat club were further subjected to RAPD PCR finger printing using Col 1, GEN 15009, OPC 9, API 2, E 2 primers. Samples observed were 1 to 15. The result of genomic digestion using standard enzymes (Table.3) is illustrated in Fig. 3.. PCR conditions of denaturation annealing followed by amplification resulted in variation of fragments seen in all the 15 cases the phylogenetic study of all the genome samples were further subjected to computational configurations using NTSYS software. The resulting patterns of dendrogram based upon PCR finger printing using RAPD analysis represent heterogeneous pattern of grouping (Fig. 4). The variation occurring in genomic cluster is related to the kind of enzymes used for RAPD process. Since the amplification of genome is irrespective of similarity and continues as random therefore the number of genomic segment amplified from any sample is not predetermined. The dendrogram shows deviation of initial two groups at 27% as well as 42% similarity sample B1 and B13 from Boat club site falls with absolute similarity, B4 and B10 also shows absolute resemblance in relation to pattern of amplification. Further B2, B8, B13, B4, B10, B15, B7, B5, B11 forms a group at 40% similarity. RAPD analysis shows B9 and B1 genomic component at 58% and 50% dissimilarity.

Conclusion

- The Upper Lake of Bhopal is a source of potable water in the city. About 40% of the population of city use this water for drinking, recreation and fisheries purposes. Due to dumping of sewage effluents, hospital wastes, other anthropogenic inputs and religious activities, the water quality of Upper Lake has been deteriorating since last few decades.
- The main purpose of this research exploration is the diversity analysis of microbial population of upper lake, Bhopal using existing molecular techniques and bioinformatics tools. In this study we evaluate the use of different DNA-based technique for characterization and study of genetic variation among different isolates /strains.

- On the basis of different physiological parameters studied, it seems that most of the species are identical; however the genomic studies indicate their diversity. The present research will help in conserve biodiversity with particular sequences and will also applicable to identify the microbial population of upper lake, Bhopal which will help in microbial testing of contamination.

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