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Isolation and Molecular Identification of few Opportunistic Pathogenic Environmental Microorganisms from River and Lakes in Bengaluru, India

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Abstract: Water used for drinking, recreational purpose and irrigation if contaminated with opportunistic pathogenic environmental microorganisms may have health implications as it propagates and disseminates human pathogens. This study aimed to isolate, identify (molecular identification) environmental microorganisms from various River and Lakes in Bengaluru, India. The water samples from Kagglipura and Avalahalli lakes, Dakshina Pinakini and Vrishabhavathi rivers were analysed for the presence of faecal indicator bacteria as well as common aquatic bacteria like Aeromonas and Pseudomonas species. Following the molecular identification of the isolates using 16S rDNA and 18S rDNA sequencing, faecal and total coliforms were detected in the untreated water samples from the Dakshina Pinakini and Vrishabhavathi river, with E.coli, Fusarium oxysporum, and Alternaria burnsii being the most prevalent genera. The water samples from the Kagglipura and Avalahalli lakes showed the presence of opportunistic pathogenic microorganisms like Aeromonas aquariorum, Klebsiella pneumoniae, Citrobacter freundii, that may significantly affect human, animal and plant health. Hence, the study highlights that the water from these River and Lakes in Bengaluru contains various opportunistic microbial pathogens. Such waters must therefore be treated suitably to meet the human requirement.

Keywords: Molecular identification, opportunistic pathogens, River and Lakes.

1. INTRODUCTION

1.1. Water

Water is a distinctive constituent. It has set the stage for the evolution of life and is an essential ingredient of our lives today it may well be the most precious resource the earth provides to humankind.
High quality of water is essential for human life for agriculture, industries, and commercial uses. Water resources have increasingly come under pressure of late due to competing and ever-increasing demands from different sectors. Where the water demand for water is increasing day by day in a logarithmic manner. What is often said that future wars will be fought for water, seems prophetic, given the depleting water resource endowment both in quality and quantity. That the groundwater resource in the country is the most stressed in the world, drives us to think of alternatives to water management. As we all know, understanding of technical aspects of water resources is essential to appreciate the limits to exploitation and technological options for judicious use of the resource.
As for higher demand, higher pollution rates and lower quality and quantity of water and contamination of hydrosphere by different microbes such as bacteria, fungi, phages, etc
Lakes and river waters are becoming highly polluted different industrial processes and biological processes why certain industrial effluents as a manner of source of nutrients for the microbes, certain pathogenic organisms utilize this nutrient thrive in it luxuriantly
Due to this reason, the lake and river waters are becoming highly pathogenic and contagious, and very undesirable for human usage.
In this experiment, I'll be isolating and using molecular identification to determine the organisms present in a few lakes and rivers present in the bengaluru district
As we are concentrating on the bengaluru district here's a brief history of lakes and rivers which used to exist in the bengaluru district.

1.2. Water pollution

Bengaluru used to have thousands of lakes in the 16th century and two prominent rivers flowing through them.

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But urbanization industrialization and encroachment of storm drains and encroachment of lakes for the building of different amenities required by the bengaluru residents has led the to loss of a large number of lakes by repurposing them. While the dakshina pinakini river has been running dry, in the place where water is run earlier now sewage from bengaluru runs through its channels, which used to originate in the Nandi hills run through different parts of rural and urban bengaluru finally reaching tamil nādu at hosur and going into the bay of bengal.
While the other river vrishabhavathi river which still flows to this point of the day is heavily polluted by untreated sewage and industrial effluents as a few industries are located on its banks where the river originates near kaadu malleshwara temple malleswara and flows through different regions of the city and finally meeting the river arkavati as one of its tributary in kanakpura.
Scope of the study: the two prominent rivers and two lakes that reside between the bengaluru urban and rural regions were in them selected for the microbiological study to determine the health and extent of pollution.

2. AIMS AND OBJECTIVES:

- The aim of the following experiment was to determine the health of following river and lakes to see if the water in them is fit for human basic needs
- The aim of the following experiment was also to learn about sangers sequencing method, pcr, bacterial 16s rdna extraction and sequencing, fungal its extraction and sequencing.

3. METHODOLOGY USED

- Sample collection
- Culturing of collected water samples using serial dilution method
- Morphological analysis of the culture plates samples to rule out any e-coli cultures
- Further culturing of selected bacterial and fungal samples for genomic dna extraction and sequencing

3.1. Sample collection

Water samples were collected from kaggalipura lake avalahalli lake dakshina pinakini river and vrishabhavathi river at select locations in sterile 50 ml screw cap tubes.

3.2. Culturing of collected water samples using serial dilution method

The collected water samples was diluted 10^{-7} and 10^{-3} to 10^{-7} 0.1 ml of the samples were added plates of nutrient agar plates and incubated for 24 hours for bacteria and 0.1 ml of a sample were added to PDA plates and incubated for 7 days for fungal colonization

3.3. Morphological study of culture plates to rule out any e-coli cultures

All the cultures on the plates were studied for morphological characters to rule out the common contamination of e coli and find specific colonies exhibiting particular characters of other bacteria and fungi.
Futher culturing of the selected bacterial colonies and fungal samples for genomic dna extraction
The selected samples where inoculated in nutrient broth medium for bacteria and Fluid Thioglycollate Medium for fungi The nutrient broth medium were incubated at 37°C for 24 hours and the Fluid Thioglycollate Medium was incubated for 7 days on a shaker incubator

3.4. Genomic dna extraction of bacteria

- The inoculated colonies were suspended in Eppendorf tubes and centrifuged at13500rpm for 10 minutes, the supernatant is discarded and the pellet is collected (this step is repeated around 2-3 times more till enough pellet has been collected for the further procedure).
- The pellet is suspended in 500µl of ste [sodium tris edta] buffer, to which 20µl of 20% sds + 10µl of proteinase-k is added.
- This mixture in tube is vortexed at 1350rpm for physical breakage of cell and kept in dry bath for 30minutes of incubation [till the pellet gets dissolved].
- After incubation, 500µl of pci [phenol chloroform isoamyl alcohol in ratio 25:24:1] is added to the same tube and subjected to centrifugation at 13500rpm for 10 minutes.
- After centrifugation, aqueous layer is collected with the help of micropipette and transferred in other fresh vial. Once transferred in fresh vials add equal volume of ci [chloroform isoamyl alcohol in 24:1 ratio]. This is again centrifuged at 13500rpm for 10minutes.
- Aqueous layer is collected in fresh 1.5ml vials and to it 20µl of sodium acetate + 60µl of glycogen and 1ml of isopropyl alcohol [ipa] is added. This mixture is then refrigerated at -4ºc for 20minutes.
- Once refrigerated, centrifuge the mixture for 10 minutes at 13500rpm and pellet is collected by carefully discarding the supernatant. The pellet is washed in 300µl of 70% ethanol and mixed thoroughly. This is again centrifuged at 13500rpm for 10minutes.

- The ethanol is thus evaporated by keeping the tubes in dry bath for 30minutes. Once the ethanol has been completely evaporated, 20µl of miliq
- Water is added to the vial and the vial is slow tapped for the dna to get suspended in it. Equal volume of dna sample and gel loading dye [around 5µl each] are mixed and prepared to subject for gel electrophoresis.

3.5. Genomic dna extraction of fungi

- The inoculated colonies were suspended in eppendorf tubes the pellet is suspended in 100µl of ste buffer, to which 20µl of 20% sds
- The contents are crushed using a pinch of glass powder and micro pestle after this 400µl of ste buffer + 10 µl ß-mercaptoethanol
- This mixture in tube is vortexed at for physical breakage of cell and kept in water bath for 1hour 30minutes of incubation at 55°c
- After incubation, 300µl of pci [phenol chloroform isoamyl alcohol in ratio 25:24:1] is added to the same tube and subjected to centrifugation at 13500rpm for 10 minutes.
- After centrifugation, aqueous layer is collected with the help of micropipette and transferred in another fresh vial. Once transferred in fresh vials add equal volume of ci [chloroform isoamyl alcohol in 24:1 ratio]. This is again centrifuged at 13500rpm for 10minutes.
- Aqueous layer is collected in fresh 1.5ml vials and to it 20µl of sodium acetate + 60µl of glycogen and 1ml of isopropyl alcohol [ipa] is added. This mixture is then refrigerated at -4ºc for 20minutes.
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- Once the ethanol has been completely evaporated, 20µl of miliq water is added to the vial and the vial is slow tapped for the dna to get suspended in it.
- Equal volume of dna sample and gel loading dye [around 5µl each] are mixed and prepared to subject for gel electrophoresis.

4. AGAROSE GEL ELECTROPHORESIS

- Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb.
- To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current applied.
- The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged anode. Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size within an agarose gel in a pattern such that the distance traveled is inversely proportional to the log of its molecular weight. The leading model for DNA movement through an agarose gel is "biased reptation", whereby the leading edge moves forward and pulls the rest of the molecule along
- The rate of migration of a DNA molecule through a gel is determined by the following:
    - size of DNA molecule.
    - agarose concentration.
    - DNA conformation.
    - voltage applied.
    - presence of ethidium bromide.
    - type of agarose and size of the pores.
    - electrophoresis buffer.
    - After separation, the DNA molecules can be visualized under UV light after staining with an appropriate dye.

5. POLYMERASE CHAIN REACTION

- Polymerase chain reaction (PCR) is the in vitro amplification of specific sequences of nucleic acid. Polymerase chain reaction (PCR), invented by scientist Kary Mullis in the early 1980s, and for which he won a Nobel Prize in 1993, allows researchers to amplify pieces of DNA by several orders of magnitude. This technique has revolutionized many aspects of current research, including DNA cloning and sequencing, functional analysis of genes, the diagnosis of hereditary or infectious diseases, the identification of genetic fingerprints, and so on. The basic components of a PCR reaction include a DNA template, primers, nucleotides, DNA polymerase, and a buffer.

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- The DNA template usually is your sample DNA, which contains the DNA region to be amplified. It could be plasmid DNA, genomic DNA, or even a small amount of tissue. The template DNA is typically given at very low concentrations in a PCR reaction.
- Primers are short oligonucleotides of DNA (typically 15–25 nucleotides) with a specific sequence that is custom synthesized on an automated DNA synthesizer. In general, the two primers match to the two ends of the segment of DNA one wants to amplify. Through complementary base pairing, the 5'-end primer matches to the top strand at one end of your segment of interest, and the other primer matches to the bottom strand at the other end.
- DNA polymerase is an enzyme complex that amplifies DNA during cell cycle in a living organism. The DNA polymerase used in a PCR reaction usually can tolerate high temperature (95°C), the temperature necessary to separate two complementary strands of DNA in a test tube. For example, the Taq polymerase purified from Thermus aquaticus, a strain of bacteria living in a hot spring, can survive near boiling temperatures, and it works quite well at 72°C.
- dNTPs - Nucleotides are the building blocks for making the DNA molecules. In PCR reactions, a mixture of four types of nucleotides (ATP, CTP, GTP, TTP; known as dNTPs) will be added. DNA polymerase grabs the complementary nucleotides that are floating in the liquid around it and attaches them to the 3 end of a primer and pairing with the template DNA.
- PCR buffers help to maintain the right pH during the reaction cycles and provide necessary ions for enzymes to work. A typical PCR buffer stock solution is provided in a 10X or 5X format; you would need to dilute it to 1X in the PCR reaction.
- Steps involved
  - DENATURATION
  - ANNEALING
  - EXTENSION

6. GENE SEQUENCING

6.1. Method of sanger sequencing

- The dna sample to be sequenced is combined in a tube with primer, dna polymerase, and dna nucleotides (datp, dttp, dgtp, and dctp). The four dye-labelled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.
- The mixture is first heated to denature the template dna (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing dna polymerase to synthesize new dna starting from the primer. Dna polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.
- This process is repeated in a number of cycles. By the time the cycling is complete, it's virtually guaranteed that a dideoxy nucleotide will have been incorporated at every single position of the target dna in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original dna. The ends of the fragments will be labelled with dyes that indicate their final nucleotide.
- After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called capillary gel electrophoresis. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the "finish line" at the end of the tube, it's illuminated by a laser, allowing the attached dye to be detected.

7. BIOINFORMATICS TOOL FOR IDENTIFICATION – BLAST

- The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. BLAST increases the speed of alignment by decreasing the search space or number of comparisons it makes. Specifically, instead of comparing every residue against each other, BLAST uses short "word" (w) segments to create alignment "seeds." BLAST is designed to create a word list from the query sequence with words of a specific length, as defined by the user. Requiring only three residues to match in order to seed an alignment means that fewer sequence regions need to be compared. Larger word sizes usually mean that there are even fewer regions to evaluate. Once an alignment is seeded, BLAST extends the alignment according to a threshold (T) that is set by the user. When performing a BLAST query, the computer extends words with a neighborhood score greater than T. A cutoff score (S) is used to select alignments over the cutoff, which means the sequences share significant homologies. If a hit is detected, then the algorithm checks whether w is contained within a longer aligned segment pair that has a cutoff score greater than or equal to S (Altschul et al., 1990). When an alignment score starts to decrease past a lower threshold score

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(X), the alignment is terminated. These and many other variables can be adjusted to either increase the speed of the algorithm or emphasize its sensitivity.

8. RESULTS:

- **dna extraction results** – after running the sample in agarose gel electrophoresis, dna bands can be seen in all the selected culture samples.
- **pcr results** – the extracted dna samples when amplified with primers specific to bacterial16s rdna and fungal 18s rdna.

Table.1

The primers use in amplification of bacteria and fungi		
Bacteria	Forward primer	Aagcaccggctaactccg
	Reverse primer	Ggcgtggcttccggagctaacgcgt
Fungi	Forward primer	Tcctccgcttattgatatgc
	Reverse primer	Gacactcaaacaggtgtacc

Table.2

The following organisms were obtained and sequenced			
Serial no	River or lake name	Bacteria	Fungi
1	Kaggalipura lake	Aeromonas aquariorum	
2	Avalahalli lake	Klebsiella pneumoniae	
3	Dakshina pinakini river	Citrobacter freundii	Fusarium oxysporum
4	Vrishabhavathi river	Citrobacter freundii	Alternaria burnsii

9. THESE ARE THE RESULTS AND THE SEQUENCE ID OBTAINED AFTER USING BLAST TOOL ON THE NCBI WEBSITE

Citrobacter freundii strain sl151, complete genome
Sequence id: cp016952.1length: 5096586number of matches: 9
Citrobacter freundii gene for 16s ribosomal rna, partial sequence, strain: jcm 24061
Sequence id: ab548826.1length: 1497number of matches: 1
Klebsiella pneumoniae strain 1756, complete genome
Sequence id: cp019219.1length: 5195816number of matches: 8
Aeromonas aquariorum antonio martinez-murcia:mdc310 16s ribosomal rna gene, partial sequence
Sequence id: jq034591.1length: 1473number of matches: 1
Alternaria sp. 16 gmf-2018 small subunit ribosomal rna gene, partial sequence; internal transcribed spacer 1 and 5.8s ribosomal rna gene, complete sequence; and internal transcribed spacer 2, partial sequence
Sequence id: mk046765.1length: 543number of matches: 1
Fusarium oxysporum strain 08ysjg 18s ribosomal rna gene, partial sequence; internal transcribed spacer 1, 5.8s ribosomal rna gene, and internal transcribed spacer 2, complete sequence; and 28s ribosomal rna gene, partial sequence
Sequence id: gu136492.1length: 567number of matches: 1

10. DISCUSSION

- In this study, we describe the a few complex and diverse bacterial and fungal organisms in various rivers and lakes of bengaluru district
- The bacterial communities were investigated in terms of their species diversity and the relative abundances of the free-living communities.
- The biodiversity of these organisms, as revealed by analysis of 16s rrna genes for bacteria and 18s rrna genes for fungi were isolated and sequenced
- The organisms were identified through sanger's sequencing method
- The bacterial species identified were mainly human pathogens and opportunistic pathogen in certain conditions could cause various infection and become fatal to humans

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- The fungal species identified were mainly plant pathogens and cause diseases to specific host

11. CONCLUSION

In this present study these organisms were isolated from kaggalipura lake, avalahalli lake, dakshina pinakini river, vrishabhavathi river and identified as aeromonas aquariorum, klebsiella pneumoniae, citrobacter freundii, fusarium oxysporum, alternaria burnsii where this identification leads to use of routine microbiological analysis of water quality is required and would be necessary as it is a standard for evaluating the quality of water intended for human usage, the frequency of source water sampling, sampling and analysis methods, the scope of microbiological analysis and the acceptable limits for the target microorganisms in water samples, especially for microbes that cause disease not only by ingestion, but also by inhalation and contact. The present study concludes that proper water quality management and continuous monitoring of benthic microbes are the key factors to reduce the bacterial load and ultimately reduce the likelihood of disease outbreak.

12. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

REFERENCES

1. American public health association, 1985: standard methods for the examination of water and wastewater, 16th ed. Apha, washington, d.c.. American
2. Bergey's manual of systematic bacteriology 2nd edition. Published by springer, new york.
3. Walczak m., changes of microbial indices of water quality in the vistula and brda rivers as a result of sewage treatment plant operation. Oceanol. And hydrobiol. Studies. Xxxvii (2), 65, 2008, doi: 10.2478/v10009-007-0049-7.
4. Ahemd W.,Sawanth S,Huygens F, Goonettilleke A, Gardner T. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. Water Research.43(19), 4918,2009.
5. Walters S P., Thebo A L, Boehm A B. Impact of Urbanization and agriculture on the occurrence of bacterial pathogens and six genes in coastal waterbodies of central California. Water Research . 45(4),1752,2011,doi:10.1016/j.waers.2010.11.032.
6. Figureas M J, Borrego J J. New Perspectives in Monitoring drinking water microbial quality. Int J Envi Res Public Health.7,4179,2010,doi:10.3390/IJERPH7124179.
7. Staradumskytė d., paulauskas a. Indicators of microbial drinking and recreational water quality. Biologija. 58 (1), 7, 2012.
8. Fonseca a., botelho c., boaventura r.a.r., vilar v.j.p. Integrated hydrological and water quality model for river management: a case study on lena river. Science of the total environment. 485, 474, 2014, doi:10.1016/j. Scitotenv.2014.03.111.
9. Fonseca a., botelho c., boaventura r.a.r., vilar v.j.p. Integrated hydrological and water quality model for river management: a case study on lena river. Science of the total environment. 485, 474, 2014, doi:10.1016/j. Scitotenv.2014.03.111.
10. (rajiv p1, vol. 1(1), 2-6, august (2012) physico chemical and microbial analysis was performed on fiver river water samples collected from various districts in western tamil nadu, india by standard methods.
11. (mohan, 2010); the physio chemical analysis of water samples from kommaghatta lake
12. International journal of scientific engineering and applied science (ijseas) - volume-1, issue-9,december 2015 issn: 2395-3470 microbial status of anjanapura lake, bangalore, karnataka latha, n and ramachandra mohan, m lake management and water quality research unit department of zoology, bangalore universi0ty, bangalore-560 056

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