

Microbial Profiling Of Cyanobacteria From VIT Lake

Swati Singh, Swati Patidar, Shweta Goyal, Arabi Mohammed Saleh

Abstract: The application of molecular biological methods to study the diversity and ecology of micro-organisms in natural environments has been practice in mid-1980. The aim of our research is to access the diversity composition and functioning of complex microbial community found in VIT Lake. Molecular ecology is a new field in which microbes can be recognized and their function can be understood at the DNA or RNA level, which is useful for constructing genetically modified microbes by recombinant DNA technology for reputed use in the environment. In this research first we will isolate cyanobacteria in lab using conventional methods like broth culture and spread plate method, then we will analyze their morphology using various staining methods and DNA and protein composition using electrophoresis method. The applications of community profiling approaches will advance our understanding of the functional role of microbial diversity in VIT Lake, controls on microbial community composition.

Keywords: r-DNA Technology, Cyanobacteria

Introduction

The aim of our research is to access the diversity composition and functioning of complex microbial community found in VIT Lake. Natural population contains a range of different bacteria type from which microbes include the prokaryotic domains of cyanobacteria as well as microscopic eukarya. The research paper provides information about how cyanobacteria are structured and the abilities which they possess that support their proliferation in aquatic ecosystem. It mainly emphasizes on cyanobacteria because of their great ecological importance in the global Carbon, oxygen and nitrogen cycle as well as their evolutionary significance in relationship to plant. Molecular methods such as electrophoresis, staining techniques are used. Limitations include questions of extraction efficiency, molecular preservation, sample handling and contamination. The focus here is on study of morphology, extraction and analysis of nucleic acid and proteins from aquatic microbial community. This approach provides an analytical tool to study, the population structure and molecular ecology of microbial communities inhabiting aquatic environmental.

Material and Methods

Collection of sample:

The sampling of microorganism of aquatic environment is done from VIT Lake. Four samples collected in plastic bags from VIT Lake from four different locations to do the analysis of different types of microorganism present in the lake and to isolate cyanobacteria from all of them.



Fig 1: Collected sample

Bacterial culture:

After collecting the sample, culturing is done. In this method, BG-11 broth was prepared by mixing 1.5gm of BG-11 media in 500ml of distilled water. Then autoclaving is done at 121°C for 20 minutes. The glassware's like boiling tubes is sterilized. From now onwards all the work is done in laminar air flow to avoid contamination. After cooling own of the prepared media BG-11, we distribute it in 12 different boiling tubes. Then add 200µl, 400µl and 600µl of the sample in each boiling tubes from different sample. Properly seal it with paraffin tape to avoid contamination. Then keep these boiling test tubes in sunlight for about 5-10 days to get the growth of microorganism. The occurrence of the microorganism seen as green particles which indicates different colonies of microorganisms. To get a particular colony, spread plate method is done. In this method, nutrient agar media is prepared by mixing 12gm of nutrient agar in 300ml of distilled water. Then autoclaving of media is done and the equipment are sterilized like Petri plates, In laminar air flow the media is pour into 12 Petri plates and mark them as sample 1 -200,400,600µl, sample 2- 200,400,600µl, sample 3- 200,400,600µl, sample 4- 200,400,600µl. After solidifying media add 1ml of sample from each boiling tube to the Petri plates. Seal it with paraffin tape to avoid contamination. Keep these Petri plates in sunlight for incubation of about 2-3 days. We analyze green color lane on the agar plate showing the growth of cyanobacteria. For isolation of cyanobacteria, streaking is done in nutrient agar media. In this method the media prepared by adding 12gm of nutrient agar in 300ml

- Swati Singh, Swati Patidar, Shweta Goyal, Arabi Mohammed Saleh
- School of Biosciences and Technology, VIT University,
- Vellore-632014, Tamil Nadu, India

of distilled water. Autoclaving is done; Petri plates are sterilized and then by using wire loop streaking is done from each sample in zigzag pattern. Seal it with paraffin tape to avoid contamination. Keep it for about 2-3 days in sunlight for incubation.

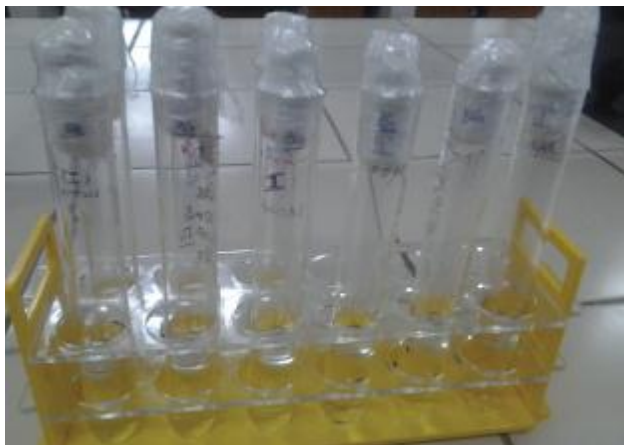


Fig 2: Samples inoculated at different concentration.



Fig 3: Cyanobacterial isolate grown in BG-11 media.



Fig 4: Cyanobacterial colonies obtained by spread plate method.

Staining methods

To analyze morphology, structure, mobility of cyanobacteria, different staining methods are done.

Simple staining:

Simple staining is done to study the morphology and the procedure is Clean and dry microscope slides thoroughly. Flame the surface in which the smear is to be spread. Flame the inoculating loop. Transfer a loop full of tap water to the flamed slide surface. Re-flame the loop making sure the entire length of the wire that will enter the tube has been heated to redness. Remove the tube cap with the fingers of the hand holding the loop. Flame the tube mouth. Touch the inoculating loop to the inside of the tube to make sure it is not so hot that it will distort the bacterial cells; then pick up a pinhead size sample of the bacterial growth without digging into the agar. Re-flame the tube mouth, replace the can, and put the tube back in the holder. Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the size of a dime. It should be a thin, even smear. Re-flame the inoculating loop to redness including the entire length that entered the tube. Allow the smear to dry thoroughly. Heat-fix the smear cautiously by passing the underside of the slide through the burner flame two or three times. Test the temperature of the slide after each pass against the back of the hand. It has been heated sufficiently when it feels hot but can still be held against the skin for several seconds. Overheating will distort the cells. Stain the smear by flooding it with one of the staining solutions and allowing it to remain covered with the stain for the time designated below. Methylene blue - 1 minute, Crystal violet - 30 seconds, Carbol fuchsin - 20 seconds. During the staining the slide may be placed on the rack or held in the fingers. At the end of the designated time rinse off the excess stain with gently running tap water. Rinse thoroughly. Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel. Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective. Choose an area of the smear in which the cells are well spread in a monolayer. Center the area to be studied, apply oil directly to the smear, and focus the smear under oil with the 100X objective cells observed.

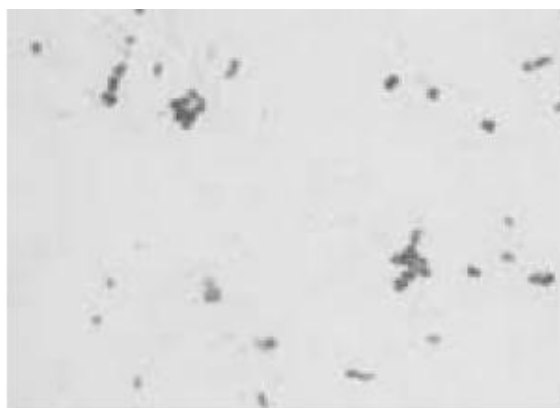


Fig 5: Simple staining.

Gram staining:

Gram staining (or Gram's method) is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a

thick layer in Gram positive bacteria. A Gram positive results in a purple/blue color while a Gram negative results in a pink/red color. Prepare and heat-fix smears. Flood the crystal violet for one minute. Pour off excess dye and wash gently in tap water. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over. Wash with tap water and drain carefully. (Do not blot.) Wash with 95% alcohol for 30 seconds. Wash with tap water at the end of the 30 seconds to stop the decolorization. Drain. Counterstain with 0.25% safranin for 30 seconds. Wash, drain, blot, and examine under oil.

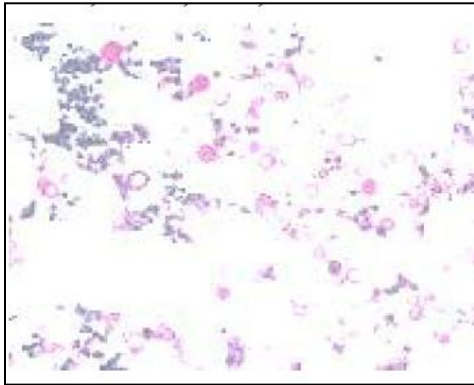


Fig 6: Gram staining.

DNA extraction method:

Current protocol for the preparation of cyanobacteria genomic DNA consist of Lysozyme/detergent lysis, followed by incubation with a non-specific protease and a series of phenol /chloroform/isoamyl alcohol extractions prior to alcohol precipitation of the nucleic acids. This procedure effectively removes contaminating proteins. Grow 50ml of liquid culture. Spin down 25-50ml of the culture and remove supernatant. Resuspend cells with 1ml of T1/10 pH 7.5 and transfer to epp. Tube. Spin down cells by micro centrifuge at room temperature for 1 min. Remove supernatant and resuspend the cells into a volume of about 400µl with T1/10E pH 7.5. Add about 150µl of sterile glass beads. Add 20µl of 10% SDS. Add 450µl of phenol: chloroform (1:1). Full-power vortex for 1min. and keep it in ice for 1min. Repeat 3 times. Spin for 15 min. In a microfuge at 4°C. Transfer the transparent supernatant (about 400µl) to an epp. Tube. Phenol extracts the supernatant once with 500µl phenol (1 min.) then chloroform extract twice / 3min. Each). Add 10% volume (40µl) of 3M NaCl, 2.5 volumes (900µl) of absolute ethanol for 3min. Keep the tube in -20°C for 30 min. to precipitate DNA/RNA. Microfuge for 10min. At 4°C discard supernatant. Wash pellet once with cold 70% ethanol. Resuspend pellet with 150µl T1/10E pH 8.0. Store at 20°C. (Usually there will be more than 1mg of DNA in 5ml of the final solution. 10µl of the solution is more than enough for one lane on a gel for southern blot). The yield of genomic DNA depends on size of the organism and age of the culture.



Fig 7: DNA extracted from cyanobacteria.

Gel electrophoresis:

Agarose gel electrophoresis is a simple and highly effective method for separating, identifying and purifying DNA fragments. Voltage applied at the ends of an agarose gel generates an electric field with a strength defined by the length of the gel and the potential difference at the ends (V/cm). DNA molecules exposed to this electric field migrate toward the anode due to the negatively charged phosphates along the DNA backbone. The protocol can be divided into 3 stages. A gel is prepared with an agarose concentration appropriate for the size of DNA fragments to be separated. The DNA samples are loaded into the sample wells and the gel is run at a voltage and for a time period that will achieve optimal separation and the gel is stained or, if ethidium bromide has been incorporated into the gel and electrophoresis buffer, visualized directly upon illumination with UV light. Prepare an adequate volume of electrophoresis buffer (TAE) to fill the electrophoresis tank and prepare the gel. Add the 350mg of electrophoresis grade agarose to a 50ml of electrophoresis buffer for constructing the gel. Melt the agarose in a microwave oven or autoclave and swirl to ensure even mixing. Melted agarose should be cooled to 55°C and add 5µl volume of ethidium bromide. Seal the gel casting platform, pour the melted agarose and insert the gel comb, making sure that no bubbles are trapped underneath the combs and all bubbles on the surface of the agarose are removed before the gel sets. After the gel has hardened, remove the tape from the open ends of the gel platform and withdraw the gel comb. Place the gel casting platform and withdraw the gel comb. Place the gel casting platform containing the gel in the electrophoresis tank. Add sufficient volume of electrophoresis buffer to cover the gel to a depth of about 1mm. Add DNA samples and molecular weight marker in wells sequentially. Set the voltage to the desired (1 to 10V/cm) to begin electrophoresis. The progress of the separation can be monitored by the migration of the dyes in the loading buffer. After 90 min. Run, turn off the power supply and observe the gel with UV transilluminator. DNA fragments should be well resolved as separate bands. Fragments size can be detected by comparing DNA molecular weight markers.



Fig 8: DNA bands observed on gel electrophoresis.

Result and Discussion

The profiling of cyanobacteria from environmental microbial samples is an important step to increase our understanding of the complex process of microbial ecology. We observe that cyanobacteria are quite small and usually unicellular, though they often grow in colonies large enough to see, they have no nucleus or internal membrane systems produce spherical colonies as much as three or four centimeters in diameter. Cyanobacteria are spherical in shape and gram negative bacteria .DNA is isolated and run on agarose gel, four bands are observed.

Conclusion

Our project concluded that the cyanobacterial family is autotroph, has spherical morphology and is gram negative. It is nitrogen fixing and also helps in balancing carbon , oxygen and nitrogen cycle. The genomic DNA is isolated from the overgrown culture of cyanobacteria and then it is run on agarose gel for obtaining fragments of DNA. After running of DNA four bands are observed.

References

- [1] Anand Nighojkar, Sadhana Nighojkar,2007. Experiments in biotechnology. 72,77,177,202.
- [2] G. Subramaniam, L. Uma, M. Sundararaman, D. Prabakaran, P. Malliga, N. Thajuddin, S. Sekar, T. Thirunala Sundari,1999. 24, 75,87.
- [3] Lansing M. Prescott,2002. 424,430.
- [4] Katherine C. Dent, John R. Stephen¹, William E. Finch-Savage,2003. Molecular profiling of microbial communities associated with seeds of Beta vulgaris subsp. Vulgaris (sugar beet). 17,18,25.
- [5] T.A Brown, Gene Cloning And DNA Analysis.189.
- [6] Barbara J MacGregor,1999. Molecular approaches to study of aquatic microbial communities. K.A. Gilbride , D.-Y. Lee , L.A. Beaudette, 2006.
- [7] Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control. Ken C. McGrath, Skye R. Thomas-Hall, Chu Ting Cheng, Lesina Leo, Andrei Alexa, Susanne Schmidt, Peer

M. Schenk,2008. Isolation and analysis of mRNA from environmental microbial communities.

- [8] Jennifer L. Kirk, Lee A. Beaudette, Miranda Hart, Peter Moutoglis, John N. Klironomos, Hung Lee, Jack T.
- [9] Trevors, 2004. Methods of studying soil microbial diversity.