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Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

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A Primitive Studies On Isolation And Cultivation Of Cyanobacteria, Gleocapsa Spp.

M. B. Dave And H. V. Sukhadia
Research Scholar. Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan
Ph.D. Supervisor, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan

Abstract:-Cyanobacteria: "True bacteria with a prokaryotic cell structure and containing chlorophyll-a(a photo - pigment characteristic of eukaryotic algae and higher plants)", are found in almost every conceivable environment, from oceans to fresh water to bare rock to soil. A procedure has been developed to isolate and cultivate Gleocapsa spp. an aquatic cyanobacterial spp. using various novel research techniques for sampling like Direct scrubbing, water scum sampling, and for Cultivation and Isolation like Capillary Transfer Technique, Transfer with help of Glass Beads, Transfer with help of Filter Paper, as a final result pure culture of Cyanobacterial spp. was obtained.

Keywords : Cyanobacteria, Gleocapsa spp.,

INTRODUCTION

Cyanobacteria, a.k.a. blue-green algae, are very common forms of photosynthetic bacteria present in most freshwater and marine systems. They are abundant throughout the world and contribute significantly to global primary productivity from which picoplanktonic marine and aridland terrestrial components account for a large proportion (García-Pichel et al. 2003). The monitoring of cyanobacteria is of growing interest in a number of research and monitoring fields and of particular interest is the monitoring of cyanobacteria as a public health risk. As the rates of eutrophication accelerate due to human impacts on aquatic ecosystems, algal blooms are becoming a more common problem. General technique for the sampling[1] given by the procedures listed is excerpted from the Department of Environmental Protection (DEP): Standard Operating Procedures (SOP) to collect aqueous samples or Cyanobacteria

A large variety of media are available for the cultivation of Cyanobacteria. But we are preferentially selected the main five medium for cultivation because the availability of the required ingredients in laboratory and these mediums fulfill our requirements for cultivation. For convenience, in these standard media (with minor modifications), are used for the maintenance[2]. Cultures are maintained[9] in the lab at 25 °C under the photosynthetic activity, 12 h light/12 h dark period of light. The isolated cultures are maintained on agar plates and in to the liquid medium[2], [11]. When sufficient growth of the subcultures has occurred, more optimal growth conditions may be investigated..

MATERIALS AND METHODS

Sea water, fresh water, and sewage water samples are collected from different regions of Gujarat (As listed fig.). There are so many methods available for the sampling of fresh water as well as marine water Cyanobacteria. Among all those techniques we have used three techniques as noted below;

1.Direct scrubbing:

This is the new technique discovered for the cyanobacteria which can grow in contact with the supporting material in moist condition. In this method samples are directly scrubbed from the solid surface i.e. rocks and walls by the mean of presterilized scalpel. But this technique should be handled with care because it may lead to breaking down of algal filaments. After the scrubbing, the cells/filaments are directly collected into the same water sample and immediately (Within 1-2 days) used for further procedure.

2.Scrubbing from the wall:

In this technique, the cyanobacteria are directly collected from the wall by scrubbing. And this sample is preserved into fresh water or Mill Q water - sterile (for 1-2 days).

3.Water scum sample. / Surface scum sample:

Water scum sample technique is the best method for the collection of Cyanobacteria and algae, having the capacity to formation bloom on to the water surface. With the help of wide mouth polycarbonate bottle or glass bottle collect surface grab sample within the top 5-10 cm of the water which is having the Cyanobacterial bloom on its surface. Do not collect the sample by skimming the surface. After samples are collected then with the help of Nicrome wire loop transfer the filaments and water sample in to the cultivation medium. This technique gives the promising results then the other which we were used.

Purification, Isolation and Cultivation

Isolation and cultivation were carried out followed by the sampling, for that we have used the following five recommended medium which were; BG-11 Medium, Castenholtz D Medium, Gorham's Medium, ASN-I Medium, Bold Basal Medium.[10]

BG – 11 Medium	
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric citrate*	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g
Trace metals	1.0 ml
Agar (if needed)	10.0 g
Distilled water	1.0 L

Trace Metals	
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg
Distilled water	1.0 L

Castenholtz D Medium		**Castenholtz Micronutrient Solution:**	
		$MnSO_4 \cdot H_2O$	2.28 g
$NaNO_3$	0.7 g	H_3BO_3	0.5 g
Na_2HPO_4	0.11 g	$ZnSO_4 \cdot 7H_2O$	0.5 g
KNO_3	0.10 g	$CoCl_2 \cdot 6H_2O$	0.025 g
$MgSO_4 \cdot 7H_2O$	0.10 g	$CuSO_4 \cdot 5H_2O$	0.025 g
Nitrilotriacetic acid	0.10 g	$Na_2MoO_4 \cdot 2H_2O$	0.025 g
$CaSO_4 \cdot 2H_2O$	0.06 g	H_2SO_4	0.5 ml
NaCl	8.0 mg	Distilled water to	1.0 L
$FeCl_3$ solution	1.0 ml	**$FeCl_3$ solution:**	
Castenholtz Micronutrient Solution	0.5 ml	$FeCl_3 \cdot 6H_2O$	2.28 g
Distilled water to	1.0 L	Distilled water	1L

Gorham's Medium(ATCC Medium no. 625)	
$NaNO_3$	496.0 mg
K_2HPO_4	39.0 mg
$MgSO_4 \cdot 7H_2O$	75.0 mg
$CaCl_2 \cdot 2H_2O$	36.0 mg
Fe citrate	6.0 mg
$Na_2SiO_3 \cdot 9H_2O$	59.0 mg
Na_2CO_3	20.0 mg
Citric acid	6.0 mg
EDTA	1.0 mg
Glass - distilled water	1.0 L

Among all above listed medium, Castenholtz D medium[8] gave luxurious growth followed by BG-11medium[3] and Gorham's medium[1]. Expected growth was not found in ASN-I medium[15], [2], which is designed for the growth of true marine Cyanobacteria. Bold Basal medium[3] gave the difficulty of moisture loss and excessive evaporation hence the last two medium were less frequently utilized. So, further work was carried on the Castenholtz D medium.

BG-11 medium was modified further due to constrain of some specific ingredients like Ferric ammonium citrate and cyclohexemide to restrict the growth of fungi. And in the Castenholtz D mediumhere we use the antibiotic-Ampicillin (100µg/ml), Streptomycin (40µg/ml) and Chlorohexidine (60µg/ml) for overcome the contamination of the autotrophic bacteria and fungi. BG-11 medium allows growth of wide variety of autotrophic bacteria and fungi[2] so; this medium was not used for further transfer. The Castenholtz D medium gives the most promising results.[4]

Cultivation

10% inoculums were inoculated in to the liquid medium. Filamentous samples were directly inoculated in the medium with the help of needle and mix thoroughly. And for the water sample and scum sample, solid medium was spreaded with the help of sterile pipettes. 0.1ml of sample was spread on to the solid medium. After inoculation flask and plates were placed in to the incubator under the regular intervals of illumination. Here we use the mainly three medium (as mentioned previously) with providing the antibiotic-Ampicillin (100µg/ml), Streptomycin (40µg/ml) and Chlorohexidine (60µg/ml) for overcome the contamination of the autotrophic bacteria and fungi. [4]

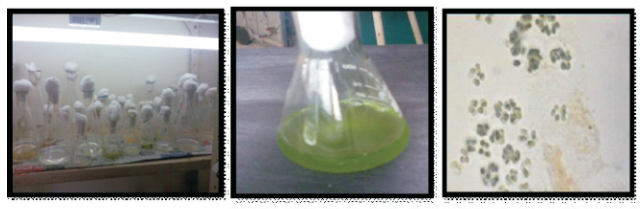
After sufficient visible growth (around 15 days) microscopy was carried out. The growth is transferred to fresh medium. For carrying out the transfer following four techniques were applied for the first time.

1.Capillary Transfer Technique: This technique is only use for transfer the organism in liquid medium. In this technique we used pre sterilized glass capillary for transfer the organism from one liquid medium to another (sterile medium). Capillary Transfer Technique was developed by our guide, in which autoclave the capillaries in one big glass tube (Sugar tube) and then aseptically one portion of capillary dip in to the medium, containing organism and transfer to another tube having the sterile medium and leave it as such. So, in such position organisms which are in the capillary providing both-nutrient as well as aeration. In this position incubates tubes and observe the growth. After two to three transfers check the purity, and transfer to liquid and solid medium. This tech has an advantage of getting a single cell entrapped in the capillaries which will grow for a short period of time. And there is a possible of getting more than one isolates in same cultivation medium. The capillaries are then again transferred to another fresh medium.

2.Serial Dilution Technique: This technique is similar to that one of we used for isolation of bacteria. In this technique we serially diluted our sample in sterile D/W tubes and from each tube we take inoculate 0.1 ml and inoculated in to sterile respective medium solid as well as liquid. Finally we selected the plates which have isolated colonies and further transfer. Among all techniques this technique gave much promising results.

3.Transfer with help of Glass Beads: This is another technique developed by our guide. It is used for the transfer of filamentous algae. In this technique, first sterilized the sugar tubes containing 5-7 glass beads and 5 ml of sterile respective medium. Samples containing filamentous algae added filaments of a sample and thourally mix the sample with help of Vertex mixture. So in this filaments were separated and we get separates cells. They were further transfer to sterile medium with above technique. So this technique is only used for the primary separation of algae from the filamentous sample.

4.Transfer with help of Filter Paper: Many cyanobacteria can only grow on any supporting materials. So filter paper acts as supporting material for the cyanobacterial cells. In this technique filter paper is depth in to the sterile Petri plate having sufficient amount of liquid medium. And inoculate were inoculated in that filter paper



Illumination | Growth of *Gleocapsa spp* in Castenholtz D medium | *Gleocapsa spp.*

RESULT & DISCUSSION:

After microscopic observations the samples were sub cultured and purified by as described in materials and methods. Liquid and solid media were used for isolation and purification. Capillary method was found to be most suitable for subsequent transfer. They were photographed by Olympus trianocular microscope. Many slides were prepared and the structure was confirmed by comparing the morphological structure with the standard Reference[1 After identification the culture was grown in BG-11 (Modified) medium containing adequate amount of Floxacin, AmipicillinandStreptomycin, to avoid the bacterial as well as fungal contamination. The culture was made free from diatoms and higher algae by using chlorohexidine, and pure culture of Gleocapsa spp. was obtained by rapid transfer of cells from liquid medium to solid medium and solid medium to again liquid medium. [4][12]

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REFERENCES

1. A.P.H.A.: Standard methods for the Examination of Water and Wastewater's, 19th Edition American Public Health Association, New York. USA.

2. Allen, M. M. 1968. Simple conditions for growth of unicellular blue green algae on plates. J. Phycol. 4:1-4.
3. Allen, M. M., and R. Y. Stanier. 1968. Selective isolation of blue-green algae from water and soil. J. Gen. Microbiol. 51:203-209. VOL. 26.
4. Amit Parikh., Datta Madamwar., 2005 partial characterization of extracellular polysaccharides from Cyanobacteria.
5. Bergmen, T., J., Philips, P., 2004. Microbial mats for multiple applications in aquaculture and bioremediation. Bioresour Technol. 94(3), 229-238.
6. Betsey Betsey Dexter Dyer, 2003 a Field Guide to Bacteria, Cornell University Press.
7. Castenholtz, R.W 1992, species usage, concept, and evolution in the Cyanobacteria (blue-green algae). Jouranl of Phycology 28; 737-745.
8. Castenholtz, R. W. 2001. Phylum BX Cyanobacteria. Oxygenic Photosynthetic Bacteria. In Bergey's Manual of Systemetic Bacteriology. Volume 1: The Archea and the deeply branching and phototropic Bacteria. Second Edition. Garrity, D.R/ Boone, and R. W. Castenholtz (eds.) springer-Verlag, Newyork.
9. Conrad W. Mullineaux 2001, Department of Biology, University College London, Mol. Microbiol. 41, 965-971.
10. D.R. BOONE, R.W. CASTENHOLZ, G.M. GARRITY (eds)Taxonomic scheme according to Bergey's Manual of Systematic Bacteriology 2nd Edition, Bergey's Manual of Systematic Bacteriology, second edition, vol. 1 (The Archaea and the deeply branching and phototrophic Bacteria), Springer-Verlag, New York, 2001.
11. De Philippis, R., C., Paperi, R., V ncenzine, M., 2001 Exopolysaccharides producting Cyanobacteria and their possible exploitation; A Review J. App. Phycol. 13, 293-299.
12. Dubois, M., Gilles, K,A., Hamilton, J.K., Rebers, P.A., Smith F., 1956. Colorimetric method for determination of sugars and related substances, Anal. Chem, 28, 350-356.
13. Farias, W. R. L., et al., J. Biol. Chem. (2000) 275; (38)29299-29307; U.S. Pat. No. 6,342,367; U.S. Pat. No. 6,969,705).
14. HOWARD D. McCURDY, Jr., AND WALTER F. HODGSON Department of Biology, University of Windsor, Windsor, Ontario, Canada. APPLIED MICROBIOLOGY, Nov. 1973, p. 682-686 Vol. 26, No. 5.
15. Jenny Degerholm, Botany dept., Stockholm university, 106 91 Stockholm Ecophysiological Characteristics Of The Baltic Sea N2-Fixing Cyanobacteria Aphanizomenon And Nodularia, 1998-2000,
16. Loffelhandt, W. and Bohnert, H. J., Molecular biology of cyanelles, In the Molecular Biology of Cyanobaceria (Ed. Bryant, D. A.) Kluwer academic publishers, Dordrecht, The Netherlands, 1977, pp. 65-89.

M. B. Dave
Research Scholar. Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan