ISOLATION AND PURIFICATION OF CYANOBACTERIA IN MARINE WATER SAMPLE AT RAMANATHAPURAM (DIST),

M. Mahadevi^{1*} and S. Azhagu Madhavan²

¹PG & Research Department of Botany & Microbiology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur – 613503, India.

²PG & Research Department of Zoology & Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur – 613503, India.

Email: mahadevibotany@gmail.com

*Corresponding Author:

Dr. M. Mahadevi Assistant Professor PG & Research Department of Botany and Microbiology A.V.V.M.Sri Pushpam College (Autonomous), Poondi – 613 503 Thanjavur Dt, Tamilnadu, India.

Abstract

In the present examination broke down for assorted variety of cyanobacteria from east coast condition and physicochemical parameters of soil were investigated from the four distinctive investigation destinations. The soil physicochemical character like pH, salinity, Electrical conductivity, Organic Carbon, Organic Matter, Available Nitrogen, Phosphorus, Zinc, Copper, Iron, Manganese, Calcium, Magnesium and Potassium were experimently performed from Pamban, Keelakkarai, Tondi and Uchipuli of Ramanathapuram district conducted. The soil has extraordinary content of nutrients were presented in Keelakkarai area when compared with other places. The isolation of cyanobacteria like Anabaena azollae, Chroococcus limneticus, Dermocarpa sp., Gloeocapsa magma, Johannesbaptistia sp., Gloeothece sp., Katagnymene sp., Microcoleus vaginatus, Myxosarcina sp., Nostoc muscorum, Oscillatoria spongeliae, Plectonema phormidiuodes, Pseudanabaena sp., Spirulina sp., Stigonema sp., Symploca sp., Synechococcus sp., Trichodesmium sp and Xenococcus sp were recorded from four different places of Pamban, Keelakkarai, Tondi and Uchipuli of Ramanathapuram district were analysed. Among the four places the Keelakkarai has maximum number of colonies and species recorded than the other places. The diversity of cyanobacteria has excellent microbial resources of our country and back bone of the marine environment.

KEY WORDS: Cyanobacteria, physicochemical parameters, marine environment.

INTRODUCTION

Cyanobacteria prokaryotic, developing, unicellular filamentous are oxygen or microorganisms among which some can fix climatic nitrogen. They are phylogenetically identified with eubacteria and green growth since cyanobacteria share the qualities of both. Green growth are eukaryotic lower plants. Both cyanobacteria and green growth show likeness in autotrophic method of sustenance through photosynthesis. Numerous cyanobacteria and green growth are encompassed by an uncommon adhesive covering around their phones or fibers. Microalgae contain a huge and assorted gathering of straightforward photoautotrophic creatures going from unicellular to multicellular structures. Since they have proficient access to water, carbon dioxide and different supplements, they are commonly progressively productive in changing over sun oriented vitality into biomass. Regardless of whether they are basic and little in structure, their development involves significant parts like starches, lipids, proteins and nucleic acids. These highlights bring the eyes from the entire distinctive field like biofuel, nourishment and medication to fall on the algal biomass. Separation and purging of cyanobacteria from biological systems are required to get societies of cyanobacteria which might be useful for lab considers in basic and handy research and to improve the information concerning microbiota of the given environment. (Sarchizian et al., 2010; Arjun et al., 2017). The initial phase in disengaging cyanobacteria should be a cautious depiction of the examining site. Notwithstanding the site area and portrayal, parameters, for example, light force, temperature, pH, and saltiness, ought to be recorded. This data isn't valuable for depicting the regular living space of the cyanobacteria to be detached yet can be fundamentally significant when planning society media and states of hatching (Vinothini et al., 2017). Techniques for the detachment and refinement of cyanobacteria have been audited by Castenholz for thermophiles, by Walsby for planktonic species, by Waterbury and Stanier for creatures from marine and hypersaline situations, and strains of differing starting point. The transformed magnifying instrument ought to have a long working separation condenser to give particularly late models, have focal points for simple disconnection and perception while giving a nitty gritty picture of the objective cell. Different example dishes or magnifying lens slides can be utilized with a rearranged magnifying instrument, yet this magnifying lens exceeds expectations in its simple utilization of multiwell plates. Compound magnifying instruments can be substantially more hard to use than analyzing and altered magnifying instruments (Hariprasath et al., 2015).

Biofuel work isn't just about finding the correct sort of biomass and transforming it into fuel, yet additionally about recognizing ecologically and financially solid uses for biofuel creation results. Biofuels focus on an a lot bigger fuel showcase thus later on will assume an inexorably significant job in keeping up vitality security (Parmar et al., 2011; Arun Baskar et al., 2018). Cyanobacteria frequently called Blue green growth, have a place with the eubacteria which uses chlorophyll, for photosynthetic nourishment creation. It contains a wide scope of natural surroundings prompts wide biodiversity contemplates as indicated by different environments (Kanagasabapathi and M. Rajan 2010). Most marine regular items have diverse useful gatherings and sub-atomic structures, like those from earthly sources. Until this point in time, marine life forms have recognized 21,800 normal items (Marin Lit. 2011; Blunt et al., 2011). The working separation between the target focal point and the example is little, making it hard to pick cells with a micropipette. Additionally, most compound magnifying instruments invert the picture, making confinement strategies progressively troublesome. The cyanobacteria's least difficult morphology is that of unicells, free-living, or encased inside an adhesive shell. Consequent improvement prompted the arrangement of a line of cells called a trichome. When the trichome is encompassed by a sheath, it is known as a fiber. It is conceivable to have more than one trichome in a fiber. The most mind boggling thallus is the stretched fiber.

The ongoing screening programs for the disclosure of bioactive mixes from green growth (counting cyanobacteria) have demonstrated that cyanobacteria speak to an undiscovered bioresource for a differing scope of auxiliary metabolites, which show extraordinary similitudes with plant and creature items. In the worldwide projects, the attention has been on recognizing their antifungal, antiviral, antibacterial, antimitotic, antihelminthic, anticoagulating, hemagglutinating and lethal metabolites (Ali et al 2008). Anthropogenic stacking of nitrogen (N) and phosphorus (P) to freshwaters and waterfront marine frameworks, is a worldwide natural issue (Smith, 2003; Smith et al., 2006). The cyanobacteria are profoundly different and the attributes that may assist them with dominating are not shared by all taxa. For instance, N2 obsession must be finished by the Nostocales and somewhat number of non-heterocystous taxa like the marine Trichodesmium. In any case, most examinations exploring the reaction of phytoplankton to changes in supplement focuses over a mind-boggling number of lakes treat cyanobacteria as one reaction gathering.. (Ptacnik et al., 2008) Bioremediation is viewed as a productive and naturally safe innovation for cheap purification of dirtied frameworks. The utilization of bioremediation utilizing indigenous microorganisms for disinfecting of water frameworks dirtied with natural contaminants gives a reasonable and economical methodology for ecological assets. Cyanobacteria as bioremediating specialists have numerous favorable circumstances over different microorganisms disconnected from soil. These incorporate their photoautotrophic nature and the capacity of certain species to fix air nitrogen.

MATERIALS AND METHODS

Collection area sample

four different places of Pamban, Keelakkarai, Tondi and Uchipuli of Ramanathapuram district were analysed.

Collection of water sample (Stainer *et al.*, 1971)

Water test were gathered from four unique destinations of east shore of marine conditions and were put away in chamber at 25 oC under 8 hours/16 hours dull/light photoperiod. For morphological investigations, the Cyanobacterial strain were developed in BG 11 medium.

Physicochemical parameters of soil samples (APHA, 1985)

Physicochemical investigation of the examples were completed by the standard strategy. The precipitation information were acquired from the meteorological focal point of Ramanathapuram shore of Tamil Nadu, east shoreline of India. The climatic temperature and water temperature were estimated by utilizing thermometer. The electronic pH pen was utilized for estimating pH from ocean water, and saltiness by ATAGO hand refractometer. Disintegrated oxygen and supplements were evaluated by (Strickland and Pearsons, 1972).

Isolation, identification and purification of Cyanobacteria

The gathered example were moved in 100ml of BG11 medium. The carafe were held under adequate light (1000 lux) and hatched in the cup under room temperature (22-28°C) with a PH of 8.2±1.After 15-18 days, green staining was found in the way of life tubes because of the development of microalgae. The cyanobacterial example was gathered and weakened utilizing sterile water to 10-3, 10-4 and 10-5 individually (0.1 mL) of the diluent was vaccinated utilizing pore plate technique. The way of life was hatched at 25±2 °C under proceed with enlightenment (3,000 lux) of light source.

Algal species were detached from four distinct destinations of marine condition. As indicated by institutionalized algal disengagement system ((Rippka et al., 1979) & #41;. Recognizable proof was finished utilizing the keys given Cyanophyta by (Desikachary, 1959). Unadulterated culture of Cyanobacteria was acquired standard planting and streaking systems (Stainer et al., 1971).

RESULT AND DISCUSSION

In the current investigation suggested that the diversity of cyanobacteria like Anabaena azollae, Chrocococcus sp., Dermocarpa sp., Gloeocapsa sp., Johannesbaptistia sp., Gloeothece sp., Katagnymene sp., Microcoleus vaginatus, Myxosarcina sp., Nostoc sp., Oscillatoria sp., Plectonema sp., Pseudanabaena sp., Spirulina sp., Stigonema sp., Symploca sp., Synechococcus sp., Trichodesmium sp and Xenococcus sp were maximum number of colonies 97 and stigonema sp isolated and identified from Keelakkarai Pamban, Tondi and Uchipuli area. Among the four placces, Keelakkarai has maximum diversity of cyanobacteria were determined than the other places. However, the Keelakkarai soil nuterient content has excellent with diversity of cyanobacteria also high number of colonies were analysed. So, nutrient content of the soil has recognized factors for the population of microbiota

S.N	Name of the parameter	Different Places			
0		Keelakkarai	Pamban	Tondi	Uchipuli
1	рН	8.0	8.0	7.9	8.0
2	Electrical conductivity (dsm-1)	0.27	0.27	0.27	0.25
3	Organic Carbon (%)	0.19	0.28	0.16	0.23
4	Organic Matter (%)	0.32	0.24	0.22	0.21
5	Available Nitrogen (mg/kg)	73.7	62.5	63.3	63.2
6	Available Phosphorus (mg/kg)	4.0	3.4	3.4	3.3
7	Available Potassium (mg/kg)	110	112	105	102
8	Available Zinc (ppm)	1.02	1.31	1.16	1.12
9	Available Copper (ppm)	0.57	0.54	0.51	0.52
10	Available Iron (ppm)	4.62	3.91	3.82	3.51
11	Available Manganese (ppm)	1.33	1.25	1.16	1.11

Table 1: Physico-substance properties of soil test of south east shore of India

12	Calcium (C. Mole Proton+/kg)	1.3	0.2	0.1	0.6
13	Magnesium(C.Mole Proton+/kg)	6.7	5.2	5.1	4.3
14	Sodium (C. Mole Proton+/kg)	1.2	1.4	1.3	1.3
15	Potassium(C. Mole Proton+/kg)	0.2	0.7	0.7	0.3

C M	Name of the cyanobacteria	Keelakkarai	Pamhan	T. I.	TT.1 * . 1*
5.N 0			1 amban	1 ondi	Uchipuli
1.	Anabaena azollae	3	7	-	-
2.	Chroococcus limneticus	9	-	7	9
3.	Dermocarpa sp	-	6	-	-
4.	Gloeocapsa magma	-	4	-	-
5.	Johannesbaptistia sp	4	-	4	3
6.	Gloeothece sp	-	7	-	-
7.	Katagnymene sp	4	-	-	-
8.	Microcoleus vaginatus	3	-	2	3
9.	Myxosarcina sp	-	2	5	-
10.	Nostoc muscorum	9	-	7	9
11.	Oscillatoria spongeliae	10	-	-	-
12.	Plectonema phormidiuodes	11	4	-	6
13.	Pseudanabaena sp	12	9	6	3
14.	Spirulina sp	7	11	8	4
15.	Stigonema sp	4	5	-	4
16.	Symploca sp	7	6	7	2
17.	Synechococcus sp	-	-	6	1
18.	Trichodesmium sp	7	-	5	3
19.	Xenococcus sp	7	7	-	4
	Total no of colony	97	68	57	51

In the recent research analysed for soil physicochemical parameters and diversity of cyanobacteria were analysed. According to the soil physicochemical parameters like pH, salinity, Electrical conductivity, Organic Carbon, Organic Matter, Available Nitrogen, Phosphorus, Zinc, Copper, Iron, Manganese, Calcium, Magnesium, Sodium and Potassium were performed in four different soils. All the four soils Keelakkarai has rich nutrients can accumulated than the other three places of **Pamban**, **Tondi** and **Uchipuli** of Ramanathapuram district. The Keelakkarai soil has more lagunes and other natural invaders adapted area. so, Keelakkarai soil rich nutrients were observed. Explored in different water test from various locales of Vapi, Valsad, Surat, Gujarat, India were screened on algal culture plate and an aggregate of 8 algal detach were confined in pure form. The unadulterated types of the disconnect were kept up in 250 ml Erlenmeyer jar containing 100 ml algal culture medium at room temperature under nonstop dull and daylight period. The morphological characteristics and its identification of cyanobacteria.

Identify the potential of protease inhibition by different genera of cyanobacteria from CentralIndia.No single species grows independently and permanently in any ecosystem, beca use all species are interlinked and nutrient cyclically transformed. Environmental physicoche micalchanges can affect specific species and cause the growth and proliferation of other speci es, resulting in the succession of several species over time. The bounty of cyanobacteria is ascribed to positive substance of oxidizable natural issue and less broke up oxygen and perception. High levels of nitrogen source in the environment are also eliminating heterocystous forms since nitrogen-free media is commonly used for the isolation and purification of heterocystous cyanobacteria. Forms the foregoing discussion, it is concluded that Physico-chemical characters together with biological monitoring provided converging lines of evidence for evaluation freshwater habitats in this case as in same as in some other studies(Vijayakumar et al., 2012). BG-11 medium has shown very significant growth for cyanobacterial isolates. For the cultivation of marine cyanobacteria, the salt concentration in the BJ-11 medium was adjusted to the level present in seawater. In another economically accessible medium, for example, Chu medium, intense basal medium green pigmentation of the segregates was lost and culture turned yellow as appeared.

Conclusion

Cyanobacteria play a key role in improving the growth of many plants when applied as biofertilizers. They are also important providers of nitrogen fertilizer in the cultivation of crops because they are photosynthetic and aquatic cyanobacteria indispensable for the environment. Due to their ability to produce oxygen, cyanobacteria played a pivotal role in changing the composition of atmospheric nitrogen into plants.

Reference

- APHA. Standard Methods for Examination of Water and Wastewater, American Public Health Association WWA, Washington, D.C, 1895.
- Arjun P, Semwal D.K, Semwal R.B, Malaisamy M, Sivaraj C, Vijayakumar S. 2017. Total Phenolic Content, Volatile Constituents and Antioxidative Effect of Coriandrum sativum, Murraya koenigii and Mentha arvensis. The Natural Products Journal, 7(1): 65-74.
- Arun Baskar M., Vimala C, Bharatiraja C, Thavaselvi P, Ramesh S, Arjun P. 2018. Biomedical applications in central nerve systems. International Journal of Pure and Applied Mathematics, 118(24): 1-12.

Desikachary TV. Cyanophyta. ICAR Monograph on Algae. ICAR. New Delhi. India, 1959.

- Ferris M.J. and Hirsch C.F., (1991). Method for isolation and purification of cyanobacteria. Appl Environ Microbiol, 57, 1448–1452.
- Hariprasath L, Jegadeesh R, Arjun P, Raaman N. 2015. In vitro propagation of Senecio candicans DC and comparative antioxidant properties of aqueous extracts of the in vivo plant and in vitro derived callus. South African Journal of Botany, 98: 134–141. histories and properties of pure culture of cyanobacteria., J Gen. Microbiol, 1979; 111:1-61.
- Kanagasabapathi, V and M.K. Rajan (2010). A preliminary survey of plankton in irrukkangudi reservoir, Virudhunagar District, T.N., India. J Phytol 2/3: 63-72.
- Parmar, A., Singh, N. K., Pandey, A., Gnansounou, E., & Madamwar, D. (2011). Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresource technology, 102(22), 10163-10172.
- Ptacnik R, Lepisto" L, Wille'n E, Brettum P, Andersen T, (2008) Quantitative responses of lake phytoplankton to eutrophication in Northern Europe. Aquat Ecol 42: 227–236.
 Available: <u>http://www.springerlink.com/</u> index/10.1007/s10452-008-9181-z. Accessed 2011 Apr 1.
- Rippka R, Deruelles J, Waterbury JB, Herdman H, Stainer SY. Generic assignments, strain
- Rippka, R. (1988). Recognition and identification of cyanobacteria. In: L. Packer and A.N. Glazer [Eds] Cyanobacteria. Methods in Enzymology,167, Academic Press,New York, 28-67.
- Sarchizian, I., & Ardelean, I. I. (2010). Axenic culture of a diazotrophic filamentous cyanobacterium isolated from mesothermal sulphurous springs. Romanian Journal of Biology-Plant Biology, 55(1), 47-53.

- Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research 10: 126–139. Available: <u>http://www.springerlink.com/content/w32259833027620p/</u>. Ac- cessed 2012 Jan 4.
- Smith VH, Joye SB, Howarth RW (2006) Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51: 351–355.
- Stanier R Y, R Kunisawa, M Mandel, and G Cohen-Bazire Purification and properties of unicellular blue-green algae (order Chroococcales). 1971 Jun; 35(2): 171–205.
- Strickland JDH, Parsons TR. A practical handbook for seawater analysis. Fisheries research board of Canada, 1972, 167- 311.
- Vijayakumar S, Jeyachandren S and Manoharan C., 2012. Studies on cyanobacterial population in industrial effluents. *J of algal biomass and utilization*.3(1):39-45.
- Vinothini K, Sri Devi M, Veronica Shalini, Sudharshan Sekar, Semwal RB, Arjun P, Semwal DK. 2017. In vitro micropropagation total phenolic content and comparative antioxidant activity of different extracts of Sesbania grandiflora (L.) Pers. Current Science, 113(6): 1142-1147.