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Assessment of physicochemical parameters and isolation of cyanobacteria from marine environment

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Abstract

In the present research of isolation, identification and physicochemical parameters of two different marine water sample of Jamboranodai and Thondiyamkadu village from Thiruvarur district. The isolation of cyanobacteria like *Arthrospira jenneri*, *Aphanocapsa koordersi*, *A. platensis*, *Gloeocapsa crepidium*, *G. gelatinosa*, *G. livida*, *G. punctata*, *G. samoensis*, *G. sanguine*, *Hyella caespitose*, *Oscillatoria acuminata*, *O. amoena*, *O. homogenea*, *O. laetevirens*, *O. minimus*, *O. pseudogeminata*, *O. schultzei*, *O. subbrevis*, *O. trichoides*, *Spirulina laxissima*, *S. meneghiniana* and *S. subtilissima* were recorded from two different places in Thiruvarur district and identified on the basis of characters was also observed. Among the two different places, the Jamboranodai village has maximum number of colonies (119) and Thondiyamkadu village has minimum at (75) were recorded. The water physicochemical properties such as temperature, pH, organic carbon, organic matter, dissolved oxygen, BOD, COD, salinity, available nitrogen, phosphorus, potassium, zinc, copper, iron, manganese and sodium, calcium, magnesium, potassium. The maximum parameters in Thondiyamkadu village were (34°C), (8.0pH), (0.74%), (0.82%), (288.26mg/kg), (47.61mg/kg), (318.17mg/kg), (0.89ppm), (0.70ppm), (6.75ppm), (3.43ppm), (3.1ml/L), (3.5ml/L), (1.9ml/L), (34%), (1.86ppm), (1.71ppm), (1.83ppm) and (0.97ppm) when compared to Jamboranodai village was recorded. The Shannon (H) and Simpson (D) diversity indexes were calculated respectively. Cyanobacteria are important for optimizing the growth of many plants when used as biofertilizers.

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Introduction

Cyanobacteria are prokaryotic, oxygen-producing, filamentous or unicellular microorganisms, some of which can fix atmospheric nitrogen. Since cyanobacteria share characteristics with both eubacteria and green growth, they are phylogenetically connected to these groups (Mahadevi and Madhavan, 2020). To get societies of cyanobacteria that might be beneficial for lab analyses in basic and practical research and to improve knowledge regarding the microbiology of the given environment, it is necessary to separate and purge cyanobacteria from biological systems (Sarchizian and Ardelean, 2010). Cyanobacteria, commonly known as "blue green growth", share space with eubacteria that produce photosynthetic food using chlorophyll. It has a vast variety of natural environments which promotes diverse biodiversity considerations as suggested by different situations (Kanagasabapathi and Rajan, 2010). Additionally, the majority of cyanobacteria identified in cave entrances that receive direct or indirect sunlight are photoautotrophs (Mulec and Kosi, 2008). Some can endure prolonged darkness because they are heterotrophs (Sarma *et al.*, 2014). Cyanobacteria produce a variety of secondary metabolites to help them survive in a variety of settings where they must survive in a harsh environment (Babic *et al.*, 2015). Based on phenotypic criteria including cell morphology, sheath characteristics, or cell ultrastructure, cyanobacterial species have been identified (Jahadarova *et al.*, 2017). Recent research have used morphological characteristics like cell size, shape, colour, type of branching, sheath characteristics, and cell contents to identify and classify cyanobacteria (Salem *et al.*, 2011; Pramannik *et al.*, 2011; Dvorak *et al.*, 2017). They are considered extreme environments due to scarcity in nutrients and oxygen level compared to the surface and the microorganisms have adapted to cave habitat conditions and are generally unique (Candiroglu and Gungor, 2017). These extremely diverse microorganisms have the potential to be a rich source of important compounds that might be used in the feed, food, nutritional, cosmetic, pharmaceutical and

even the fuel industries (Olaizola, 2003). One of the most suitable, environmentally beneficial, easily accessible, and alternative sources of natural fertilizers or biofertilizers is cyanobacteria (Suresh *et al.*, 2019). The majority of environments on earth are home to cyanobacteria which are significant primary producers (Wasmund, 1997). Metal ions are extracted from the environment by microalgae, which store them in various cytoplasmic structures and use them as important nutrients in their metabolic processes (de-Bashan and Bashan, 2010). Microorganisms including such cyanobacteria and microalgae are emitted from water reservoirs or remitted from other surfaces to the atmosphere depending on the current weather conditions (e.g., wind speed, wind direction, temperature, air humidity). The method works best when ocean primary productivity is at its highest (Rosas *et al.*, 1989; Singh *et al.*, 2018; Kinga *et al.*, 2022). Cyanobacteria populate the planet's largest ecosystem and can be found in a variety of habitats including freshwater lakes, ponds, marine shores, and the open ocean (Shaden *et al.*, 2020). However, cyanobacterial hydrogen which is already on the market was thought to be a potential alternative energy source (Ananyev *et al.*, 2012). Numerous studies have demonstrated that cyanobacteria create substances that have growing medicinal and biotechnological interest, have applications in human health that have a variety of biological functions and can be used as dietary supplements (Singh *et al.*, 2017). The physicochemical parameters give information about the local environment. Multivariate methods have been applied recently to evaluate the level of seawater contamination in coastal environments (Krupa *et al.*, 2022). Even despite their a simple and small structure, they are made up of vital substances like lipids, proteins, carbohydrates, and nucleic acids (Karthika and Muruganandam, 2019). They are one of the important coastal resources and an important and vital part of the microbiota in the tropical mangrove ecosystem. They colonise any submerged surface of sediments, mangrove roots, aerial roots, branches, and trunk (Zuberer and Silver, 1978; Kathiresan and Bingham, 2001; Palaniselvam and Kathiresan, 2002).

Chroococcales are classified based on the type of cell division, the polarity of cells and colonies, the form and structure of a colony, formation of various types of mucilaginous strands, layers and the position of cells in a colony (Ram and Paul, 2021). In the present investigation was aimed to study diversity of different two sites of cyanobacteria in marine water sample and physic-chemical properties were analysed.

Materials and methods

Collection of samples

Water samples were collected from two different sites of Jamboranodai and Thondiyamkadu village from Thiruvavur district of Tamil Nadu, India. It was kept in a chamber at 25°C with an 8-hour dark/light cycle. BG 11 medium was used to culture the cyanobacterial strain for morphological research (Stanier *et al.*, 1971).

Isolation and identification of cyanobacteria

Transferring the collected sample into 100ml of BG11 medium. The flasks were maintained in an environment with enough light (1000 lux) and incubated at ambient temperature (22–28°C) with a PH of 8.2±1. The development of microalgae caused the culture tubes to turn green after 15 to 18 days. The cyanobacterial samples were obtained, diluted in sterile water to 10⁻³, 10⁻⁴, and 10⁻⁵ respectively and then inoculated using the pour plate method with (0.1 mL) of the diluent. The culture was incubated at 25°C with constant illumination from a light source producing 3,000 lux. Two different sea environments were used to isolate various types of algae. Based on the standard algal isolation were performed (Rippka *et al.*, 1979).

Identification was done using the keys of cyanophyta by Desikachary (Desikachary, 1959). Pure culture of Cyanobacteria was obtained standard planting and streaking techniques (Stanier *et al.*, 1971).

Diversity analysis

Total number of cyanobacterial strains was identified and quantified in order to estimate the diversity and richness of each study region.

1. Shannon Index of Diversity: The values of diversity has been calculated as follows (Shannon and Weaver, 1949). $H = -\sum P_i \ln P_i$

2. Simpson Index of Diversity: Simpson index calculated using equation mentioned in (Simpson, 1949) as the following: $D = 1/\sum (P_i)^2$

Physico-chemical parameters of water samples

The physicochemical analysis was performed using the standard methods (APHA, 1895). The Jamboranodai and Thondiyamkadu village from Thiruvavur district of Tamil Nadu, provided as the meteorological focal point from which the information about precipitation was recorded. By using a thermometer, the ambient temperature and water temperature were estimated. The electronic pH pen was used to estimate the pH of ocean water using an ATAGO hand refractometer. Supplements and disintegrated oxygen were evaluated (APHA, 1895; Hichem *et al.*, 2024).

Statistical analysis

The most popular diversity indices for determining the species variety of a region are those developed by Shannon (H) and Simpson (D) using Microsoft Excel 2007.

Results and discussion

Isolation and identification of cyanobacteria

Agar plating techniques are one of the most popular methods for isolating microalgae because they are simple to use and consistently produce pure isolates. Phytoplankton isolation involves a lot of time and effort, yet it is important for the research and development of microalgae for any commercial applications. The first attempt to isolate regional microalgae species from several coastal regions in Kendari, Southeast Sulawesi, Indonesia (Strickland and Parsons, 1972). Some types of algae can become tolerant to the hazardous substances present in their environment.

According to different strains of algae isolated under laboratory conditions and in the natural environment had quite different responses to metal (Andersen and

Kawachi, 2005). Therefore, the diversity of algae and cyanobacteria in two polluted water bodies was extensively investigated in this study with a significant abundance of species representing Chlorophyta and Cyanophyta (Pantastico and Suayan, 1974). The ability of *Oscillatoria* species to endure poor environmental circumstances and their capacity to retain phosphates and nitrogen may be responsible for their domination (Chukwu, 2007).

Table 1. Isolation and identification of cyanobacteria from marine water samples of Jamboranodai area and Thondiyamkadu area

Name of the microalgae	Different places (CFU/ml)	
	Jamboranodai village	Thondiyamkadu village
<i>Arthrospira jenneri</i>	03	02
<i>Aphanocapsa koordersi</i>	07	-
<i>A. platensis</i>	04	07
<i>Gloeocapsa crepidium</i>	13	09
<i>G. gelatinosa</i>	-	07
<i>G. livida</i>	06	-
<i>G. punctata</i>	03	08
<i>G. samoensis</i>	-	01
<i>G. sanguine</i>	09	-
<i>Hyella caespitosa</i>	08	-
<i>Oscillatoria acuminata</i>	11	03
<i>O. amoena</i>	-	09
<i>O. homogenea</i>	04	-
<i>O. laetevirens</i>	12	-
<i>O. minimus</i>	-	05
<i>O. pseudogeminata</i>	09	-
<i>O. schultzi</i>	-	05
<i>O. subbrevis</i>	04	01
<i>O. trichoides</i>	12	06
<i>Spirulina laxissima</i>	06	10
<i>S. meneghiniana</i>	-	02
<i>S. subtilissima</i>	08	-
Total no of colonies	119	75
Total no of species	16	14

In the current study, the diversity of cyanobacteria including *Arthrospira jenneri*, *Aphanocapsa koordersi*, *A. platensis*, *Gloeocapsa crepidium*, *G. gelatinosa*, *G. livida*, *G. punctata*, *G. samoensis*, *G. sanguine*, *Hyella caespitosa*, *Oscillatoria acuminata*, *O. amoena*, *O. homogenea*, *O. laetevirens*, *O. minimus*, *O. pseudogeminata*, *O. schultzi*, *O. subbrevis*, *O. trichoides*, *Spirulina laxissima*, *S. meneghiniana* and *S. subtilissima* were recorded (Table 1). In the Jamboranodai area, 119 colonies are present at their maximum when

compared with Thondiyamkadu area. *Arthrospira jenneri*, *A. platensis*, *Oscillatoria acuminata*, *O. subbrevis*, *O. trichoides*, *Gloeocapsa crepidium*, and *Spirulina laxissima* isolated from mainly presented at both two different sites of Jamboranodai and Thondiyamkadu village. Among the two places, Jamboranodai village has maximum diversity of cyanobacteria was determined than the Thondiyamkadu village. However, the cyanobacteria diversity and quantity of analysed colonies in the Jamboranodai village. Therefore, the water sample nutrient content has been recognized as a role in the population of microorganisms.

The eight cyanobacterial species utilized in this study's morphological characters. The cyanobacteria isolate was identified in a Vapi water sample. Eight cyanobacterial morphotypes with heterocystous and non-heterocystous morphology were observed (Okechukwu, 2009). In the present investigation, totally 22 cyanobacteria are presented in the two sites of Jamboranodai and Thondiyamkadu village were identified (Table 1). The mostly presented in the Jamboranodai village was 16 species and Thondiyamkadu village was 14 species were analyzed. The *Gloeocapsa crepidium* and *Spirulina laxissima* are almost evenly presented in both two sites of Jamboranodai and Thondiyamkadu village.

Diversity analysis

The Shannon Index values in the study are greater than those found in India by (Mayur *et al.*, 2017; Prasanna, 2007). In the present study, the highest values of Shannon and Simpson diversity indices was 2.673 H and 0.933 D respectively in the Jamboranodai village (Table 2).

Table 2. Shannon and Simpson diversity of cyanobacteria from marine water samples of Jamboranodai area and Thondiyamkadu area

Marine water indices	Jamboranodai area	Thondiyamkadu area
Shannon diversity (H)	2.673	2.459
Simpson diversity (D)	0.933	0.918

Physico-chemical parameters of water samples

Under certain environment conditions, rivers and sewage systems transport nutrients to the coastal regions (Xu, 1989). The nitrogen-free media is frequently employed for the isolation and purification of heterocystous cyanobacteria, high amounts of nitrogen sources in the environment are also eliminating heterocystous forms. The physico-chemical characteristics and biological monitoring provided convergent lines of evidence for evaluating freshwater environments in this case, as well as in some other investigations (Vijayakumar *et al.*, 2012). Similar results showing variations in the distribution of the cyanobacterial population dependent on the physico-chemical parameters were found in the studies of (Jeyachitra *et al.*, 2013). Remediation technologies are the strategic marine environmental quality management that may be beneficial for this investigation. There are certain technologies, such as bioremediation or phytoremediation, to clean up the contaminated waters. Biological technology known as "bioremediation" uses naturally occurring living organisms to speed up the biodegradation of organic and heavy metal pollutants (Shah, 2014; Jayaprabakar *et al.*, 2024). Phytoremediation in contrast hand, is the use of green plants to clean up diverse media such as soil, water or sediment, that have been contaminated with various chemicals both organic and inorganic and that interact with microbes (ITRC, 2001). In every mangrove environment, marine cyanobacteria are an essential and crucial component of the microbiology (Silambarasan *et al.*, 2012). The presence of these toxins can make it difficult to use water for a number of reasons since they have adverse impacts on society as a whole the environment, and public health in addition to altering the flavour of treated water (Maria *et al.*, 2023).

In the present research suggested that the, physicochemical parameters of the water samples were analysed. According to physicochemical parameters like temperature, pH, organic carbon, organic matter, available nitrogen, phosphorus,

potassium, zinc, copper, iron and manganese, dissolved oxygen, BOD, COD, salinity, sodium, calcium, magnesium and potassium were performed on two various water samples. The Thondiyamkadu village has a higher capacity to accumulate nutrients than the Jamboranodai village. The maximum parameters (Table 3) in Thondiyamkadu village were (34°C), (8.0pH), (0.74%), (0.82%), (288.26mg/kg), (47.61mg/kg), (318.17mg/kg), (0.89ppm), (0.70ppm), (6.75ppm), (3.43ppm), (3.1ml/L), (3.5ml/L), (1.9ml/L), (34%), (1.86ppm), (1.71ppm), (1.83ppm) and (0.97ppm) when compared to Jamboranodai village was recorded. The micronutrients like calcium, magnesium and potassium were analysed maximum at both two water samples. The minimum organic carbon, organic matter, available zinc and copper were observed in two water samples respectively. When compared to the Jamboranodai village, the water of Thondiyamkaduvillage has an extraordinarily high nutritional content.

Table 3. Analysis of physicochemical parameters of different water samples of Jamboranodai village and Thondiyamkadu village

Physicochemical parameters	Jamboranodai village	Thondiyamkadu village
Temperature (°C)	30.6	34
pH	7.8	8.0
Organic carbon (%)	0.63	0.74
Organic matter (%)	0.75	0.82
Available nitrogen (mg/kg)	267.12	288.26
Available phosphorus (mg/kg)	36.19	47.61
Available potassium (mg/kg)	241.06	318.17
Available Zinc (ppm)	0.77	0.89
Available Copper (ppm)	0.54	0.70
Available Iron (ppm)	6.13	6.75
Available Manganese (ppm)	2.91	3.43
Dissolved oxygen (ml/L)	3.4	3.1
BOD (ml/L)	2.8	3.5
COD (ml/L)	1.6	1.9
Salinity (%)	31	34
Sodium (ppm)	1.71	1.86
Calcium (ppm)	1.42	1.71
Magnesium (ppm)	1.67	1.83
Potassium (ppm)	0.81	0.97

Conclusion

The research work available by this study indicated that the evaluated utilized as biofertilizers, cyanobacteria are essential for improving the growth of many plants. As important aquatic and photosynthetic cyanobacteria for the environment and they are also major nitrogen fertiliser providers for the cultivation of crops. Cyanobacteria were significant in transforming the composition of atmospheric nitrogen into plants because of their capacity to produce oxygen. Food, energy and secondary metabolites with nutritional, cosmetic, and therapeutic values can all be made from cyanobacterial biomass. As a result, cyanobacterial farming is recommended as a sustainable agricultural method that can yield biomass with a very high responsible for conversion of environment. As essential aquatic and photosynthetic cyanobacteria for the environment, they are also significant nitrogen fertiliser providers for the cultivation of crops. Cyanobacteria were essential in transforming the composition of atmospheric nitrogen into plants due to their ability to produce oxygen. Some parameters had greater values than expected, indicating that remediation actions were needed to address such locations in order to improve the environmental quality. The results of the physico-chemical analysis showed that there was no significant fluctuation in the values and that these parameters had little influence on the occurrence and distribution of cyanobacterial general populations. The assimilation of carbon into organic compounds is the results of a complex series of enzymatically regulated chemical reactions. The cyanobacterial diversity will focused in CO₂ fixation and reductive from the environment and suitable candidature for sustainable reductive and carbon acquisition of CO₂ and balancing conditions.

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