ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 06 | June - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IDENTIFICATION OF AN INDUSTRIALLY IMPORTANT SPECIES OF CYANOBACTERIA FROM THE WATER BODY OF RANCHI DISTRICT OF JHARKHAND.

Biological Science

Deepak Kumar* Bunshi Bioscience Pvt Ltd, Argora-Kathal Pundag, Ranchi, PIN-834004 Jharkhand, India. *Corresponding Author

Rahul Anand RKDF University, Ranchi.

Ankita Kumari Bunshi Bioscience Pvt Ltd, Argora-Kathal Pundag, Ranchi, PIN-834004 Jharkhand, India.

ABSTRACT

Cyanobacteria or the blue green algae are the most diverse group of microbes with multiple applications in various industries. Different species of cyanobacteria can be used to find solutions to the major problems like global warming, organic farming, clean energy source, source of therapeutic drugs etc. Ranchi, the capital city of Jharkhand is blessed with many water bodies like lakes, rivers, reservoirs and ponds. These water bodies are the home of many cyanobacteria species that can be applied for industrial application. The current study aims to identify industrially important strains of cyanobacteria using 16S rRNA gene sequencing.

KEYWORDS

Cyanobacteria, environment, industry, bioactive compounds, blue green algae, 16S rRNA gene sequence.

INTRODUCTION:

Cyanobacteria or the blue green algae are considered to be the oldest inhabitant microorganisms of the Earth. These are one of the most diverse groups of photosynthetic microbes that are present in almost every terrain of the planet. The habitats of cyanobacteria range from terrestrial, fresh-water ponds, lakes, reservoirs and oceans to extreme environments of Arctic Circle and Antarctica to geothermal habitats like hot springs of Yellow Stone National Park, US (Gaysina *et al*., 2019, Ward and Castenholz, 2000). Total number of species of cyanobacteria discovered till today is estimated to be ranging from 2,000 to 8,000 (Nabout *et al*., 2013) which come in morphologically diverse shapes and sizes including unicellular (*Synechococcus sp.*), filamentous (*Nostoc sp.*), and spiral (*Spirullina sp.*), planktonic (*Cyanothece*) and colonial (*Merismopedia*). These diverse autotrophs are responsible for many environmental phenomenon such as Nitrogen fixation, CO_2 assimilation, oxygenic photosynthesis etc.

At present, when the world is facing multiple environmental challenges, cyanobacteria can prove to be the answer of some of the major problems. Different species of cyanobacteria can be used to generate clean energy by biofuel and bioethanol production, reducing carbon footprint by CO_2 assimilation (Savakis and Hellingwerk, 2015), replacing chemical fertilizers with cyanobacteria based biofertilizer (Pawar *et al*., 2014), thereby increasing the crop yield and soil health at the same time. In a review, (Nyok-Sean *et al*., 2015) role of certain species of cyanobacteria as cell factories has been extensively discussed. These wonder organisms have the ability to produce bioactive compounds with antimicrobial properties and industrially important high value products such as pigments, vitamins and enzymes that can be used in medical and agriculture sector.

Different species of cyanobacteria can be utilized for different industrial applications. For the purpose of categorizing different species it is important to identify them. Since, morphological identification can sometimes create confusion, it is necessary to genetically identify the different species of cyanobacteria for better application in industries. The most widely used method of genetic identification is based on the detection of 16S rRNA gene sequence. It is a ubiquitously present sequence in every bacterium and archaea. The bacterial ribosome cannot translate mRNA without its 16S rRNA subunit. Since, these genes are essential, they are highly conserved along with variable sequences. The conserved sequences can be used to design primers to target all bacteria, but they can amplify the 16S rRNA gene through the variable region in which differences in the sequence of bases allow for determination of various species.

The current study aims to identify industrially important species of cyanobacteria by 16S rRNA gene sequencing.

MATERIALS AND METHODS:

Sample Collection: Cyanobacteria samples were collected from Ranchi Lake (23.368220, 85.317488) in Ranchi district of Jharkhand. The sample was transported to the lab and stored by adding BG12 culture medium.

Establishing pure culture: The crude sample was purified by multiple serial dilutions technique. The highly diluted sample was spread on BG12 media and incubated at room temperature inside a light chamber with 12 hours light and 12 hours dark effect.

A thin filament of cyanobacteria on the agar plate was carefully scooped out and inoculated in BG12 liquid media and incubated under identical conditions with shaking.

Microscopic observation was taken every 72 hours to check the purity of the isolate.

DNA Isolation: Genomic DNA was isolated from pure culture of filamentous cyanobacteria using the DNA Isolation Kit from Bunshi Bioscience. The isolated DNA was quantified using Agarose Gel Method.

16S rRNA PCR: The genomic DNA was subjected to PCR amplification using universal primer set for 16S rRNA gene. The reaction contained 1X assay buffer, 2.5 pM concentration of forward and reverse primers, 30ng template DNA, 1 mM $MgCl_2$ and 200µM dNTPs and 3U Taq DNA Polymerase (Bangalore Genei) in a 20 µL reaction. The reaction was placed in BioEra thermal cycler for the PCR program to run.

PCR amplicon sequencing: The PCR band was carefully cut out from the agarose gel, purified and sent for sequencing to Barcode Bioscience, Bangalore.

RESULTS AND DISCUSSIONS:

The microscopic observation of the cyanobacteria culture revealed that the culture obtained was pure and has no trace of contamination. The PCR reaction yielded an amplification of 1.5kb bp (Figure – 1).

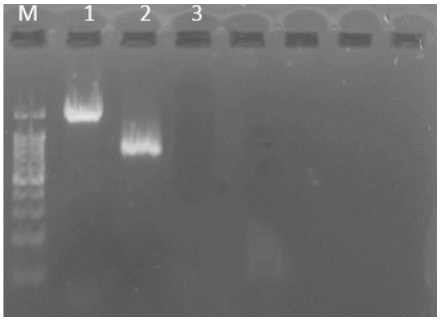

Figure – 1: M – Marker, Lane 1 – 1.5 kb amplification of 16S rRNA gene, Lane 2 – Positive Control, Lane 3 – Negative control

Sequencing – The sequencing of the PCR amplicon and analysis on NCBI database revealed the identity of the cyanobacteria to be *Leptolyngbya sp.* with 90.7% identity (Figure – 2).

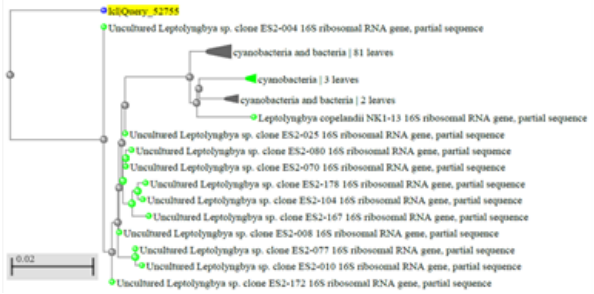

Figure – 2: Distance Tree of the identified *Leptolyngbya sp.* from Ranchi.

Leptolyngbya sp. is one of the most investigated species that can be utilized for multiple industrial applications. It is generally found in fresh water bodies, oceans, and soil and abundantly found in rice paddies (Song *et al*., 2005). It is one of the few species of cyanobacteria that can be used for multiple applications. It can be used for the treatment of waste water from agriculture and industry and at the same time biomass production with high lipid content that can be used for biomass production (Tsolcha *et al*., 2018). *Leptolyngbya sp.* is a source of many high value biomolecules such as phycocyanin pigment. Phycocyanin is extensively used in biotechnological and pharmaceutical industries. Phycocyanin content in *Leptolyngbya* sp. is higher and purer than the current commercial source, *Arthrospira platensis* (Schipper *et al*., 2020). Phycocyanin also has the potential of anti-aging and proteostasis-suppressive activities. It has been reported that phycocyanin treatment, can increase the life span of wild-type *C. elegans* by up to 22.51% (Singh *et al*., 2016). It is rich in antioxidants and can be helpful in many illnesses (Trabelsi *et al*., 2016). *Leptolyngbya sp.* can prove to be effective against drug resistant pathogens as it is an excellent source of antibacterial compounds effective against multidrug-resistant clinical pathogens, including Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus* (Tyagi S and Singh R. K., 2020).

CONCLUSION:

The current study confirms the presence of *Leptolyngbya sp.* in the water bodies of Ranchi. Thus, it can be concluded that the climate of the city is conductive for the growth of the species. Further research needs to be done in order to achieve a large scale biomass production of *Leptolyngbya sp.* so that it can be used for industrial applications discussed above.

REFERENCES:

1. Burja A M, Banaigs B, Abou-Mansour E, Grant Burgess J, and Wright P C (2001) "Marine cyanobacteria—a prolific source of natural products," *Tetrahedron*, 57(46) 9347–9377.
2. Gaysina L A, Saraf A, Singh P (2019) Cyanobacteria in Diverse Habitats. *Cyanobacteria,* 1–28.
3. Nabout J C, da Silva Rocha B, Carneiro F M, Sant'Anna C L (2013) How many species of Cyanobacteria are there? Using a discovery curve to predict the species number. *Biodiversity and Conservation,* 22(12), 2907–2918.
4. Pawar G R, Deepak-Kumar, Matharu J K (2014) The Nitrogen Fixation Capability of Cyanobacteria-A Natural Source as A Bio-Fertilizer For Arachis Hypogaea. Indian Journal of Applied Research, Vol. 4, Issue 5.
5. Savakis P and Hellingwerf K J (2015) Engineering cyanobacteria for direct biofuel production from CO 2. *Current Opinion in Biotechnology,* 33, 8–14.
6. Schipper K, Fortunati F, Oostlander P C, Al Muraikhi M, Al Jabri H M S J, Wij_els R H, Barbosa MJ (2020) Production of phycocyanin by Leptolyngbya sp. in desert environments. *Algal Research*, 47, 101875
7. Singh N K, Sonani R R, Awasthi A, Prasad B, Patel A R, Kumar J, Madamwar D (2016) Phycocyanin moderates aging and proteotoxicity in caenorhabditis elegans. *Journal of Applied Phycology*, 28, 2407–2417.
8. Song T Y, Martensson L, Eriksson T, Zheng W W, & Rasmussen U (2005) Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *FEMS Microbiology Ecology, 54*(1), 131-140.
9. Trabelsi L, Mnari A, Abdel-Daim M M, Abid-Essafi S, Aleya L (2016) Therapeutic properties in Tunisian hot springs: first evidence of phenolic compounds in the cyanobacterium Leptolyngbya sp. biomass, capsular polysaccharides and releasing polysaccharides. *BMC complementary and alternative medicine*, *16*(1), 515.
10. Tsolcha O N, Tekerlekopoulou A G, Akratos C S, Antonopoulou G, Aggelis G, Genitsaris S, Moustaka-Gouni M, Vayenas D V (2018) A Leptolyngbya-based microbial consortium for agro-industrial wastewaters treatment and biodiesel production. *Environmental Science and Pollution Research,* **25,** 17957–17966.
11. Tyagi S and Singh R K (2020) Chemical profile of the antibacterial component from *Leptolyngbya sp.* HNBGU 002 isolated from a hot spring of Garhwal Himalaya. *International Journal of Pharmaceutical Sciences and Research*. 11(10): 5225-38.
12. Ward D M, Castenholz R W (2000) Cyanobacteria in Geothermal Habitats. In: Whitton B A, Potts M (eds) The Ecology of Cyanobacteria. *Springer*, Dordrecht.
13. Whittonand M B A (2000) Potts, "Introduction to the cyanobacteria," in *The Ecology of Cyanobacteria: Their Diversity in Time and Space*, B. A. Whitton and M. Potts, Eds., pp. 1–11, Kluwer Academic, Dordrecht, The Netherlands