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GENETIC IDENTIFICATION OF CYANOBACTERIA USING PCR AMPLIFICATION OF 16S RRNA GENE

Biotechnology

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ABSTRACT

Identification of cyanobacteria, a diverse group of photosynthetic prokaryotes, plays a crucial role in understanding their ecological and physiological characteristics. One widely used approach for cyanobacteria identification is the analysis of the 16S rRNA gene, which provides valuable economical growth in industrial areas such that the production of bioplastic, biofuels, biofertilizer, and food nutraceutical and pharmaceutics .Cyanobacteria is widely used in agricultural and health sectors. One of the favorable resources is cyanobacteria also known as blue green algae. The NIF gene, also known as Nitrogen Fixation, is an essential gene involved in the process of nitrogen fixation in certain bacteria. The different strains of cyanobacteria which provides the economical growth in associated industries and nutritional values to undernourished society. The 16S rRNA gene sequences obtained from cyanobacterial isolates or environmental samples are compared to a reference database, enabling the determination of their taxonomic affiliation and phylogenetic placement. The analysis of the 16S rRNA gene has proven to be a powerful tool in deciphering the diversity and distribution of cyanobacteria, aiding in ecological studies. The NIF gene plays a vital role in biological nitrogen fixation and can be used as a biofertilizer to enhance nitrogen availability in soil for industries associated with agriculture. For example, the effects of a biofertilizer containing nitrogen-fixing bacteria on wheat, soya bean, growth. We have also discussed the challenges that hinder such development at an industrial level and ways to overcome such obstacles.

KEYWORDS

cyanobacteria, genetic identification, 16S rRNA, nitrogen fixation, etc.

INTRODUCTION

Cyanobacteria, or blue-green algae, are ancient photosynthetic bacteria that shaped Earth's atmosphere by producing oxygen over 3.5 billion years. They inhabit diverse environments and are vital for nitrogen cycling. While they are primary producers, their blooms can disrupt ecosystems. Understanding their biology is crucial for ecosystem management and their biotechnological potential (Whiton B A 2012). The nif gene is essential for bacteria to convert atmospheric nitrogen into ammonia using nitrogenase. Regulation depends on nitrogen and oxygen, with nif A activating it during nitrogen limitation. This knowledge benefits agriculture and sustainable farming. Cyanobacterial biofertilizers are environmentally friendly alternatives to chemical fertilizers. They consist of cyanobacteria, such as Anabaena and Nostoc, which have the ability to fix atmospheric nitrogen into a form that plants can use. These biofertilizers enhance soil fertility, improve plant growth, and reduce the need for synthetic nitrogen fertilizers, which can have negative environmental impacts (El-Sheekh and Aboul-Dahab, 2003). Cyanobacteria produce a variety of bioactive compounds with significant pharmaceutical potential. One notable group of compounds is cyanotoxins. Cyanotoxins are secondary metabolites that are not only harmful to aquatic life but also exhibit potential pharmacological properties, including anticancer, antiviral, and immunosuppressive activities. They have generated interest in the field of drug discovery due to their unique chemical structures and potential therapeutic applications (Borowitzka and Brown, 1974). Cyanobacteria have been investigated for their potential to produce various pharmaceutical compounds, one of which is the production of bioactive secondary metabolites. An example of a pharmaceutical product derived from cyanobacteria is Lyngbyatoxin, which is being explored for its potential in drug development (Sivonen and Leikoski, 2006). A diverse group of photosynthetic bacteria with intricate genomic taxonomy. They have been categorized into various taxonomic groups based on molecular data, including 16S rRNA and whole-genome sequencing. These taxonomic classifications are crucial for understanding the diversity and evolution of cyanobacteria (Komárek et al., 2014). Cyanobacteria also find industrial applications in wastewater treatment, pharmaceuticals, and as a source of high-value compounds like pigments and antioxidants (Rastogi et al., 2010). Cyanobacteria have gained significant attention in recent years for their potential in industrial applications. These microorganisms offer various advantages, including rapid growth, high biomass production, and the ability to produce valuable compounds. One notable area of industrial development is in biofuel production, where cyanobacteria are explored as a source of biofuels, such as biohydrogen and biodiesel (Dutta, D., De, D., & Chaudhuri, S. (2009).

MATERIALAND METHODS

DNA Isolation: Genomic DNA from the cyanobacterial strain was isolated using genomic DNA isolation Kit from Bunshi Bioscience.

Polymerase Chain Reaction: The next stage involves amplification of the conserved domain of 16S rRNA gene region. Universal primer set for 16S rRNA gene was used for the amplification. The PCR reaction had the following key steps: initial denaturation at 94°C for 5 minutes, followed by a cycle of three steps, denaturation at 94°C for 50 seconds, annealing at 52°C for 45 seconds and extension at 72°C for 1 minute. The reaction ended with a final extension at 72°C for 5 minutes. The amplified PCR product was subsequently analyzed on 1.5% agarose gel.

PCR Purification: The PCR reaction was purified using the PCR cleanup kit. The purified amplicon was processed for sequencing using equipment provided by Applied Biosystems, USA, to obtain the final DNA sequence.

The sequence obtained was analyzed using the sequence alignment tools available on the NCBI Database.

RESULT

PCR amplification of genomic DNA from the pure culture of cyanobacteria species (Figure: 1) yielded 800bp amplicon. Analysis of the conserved domain regions of 16S rRNA of the cyanobacteria species revealed its identity to be Synechococcus sp.

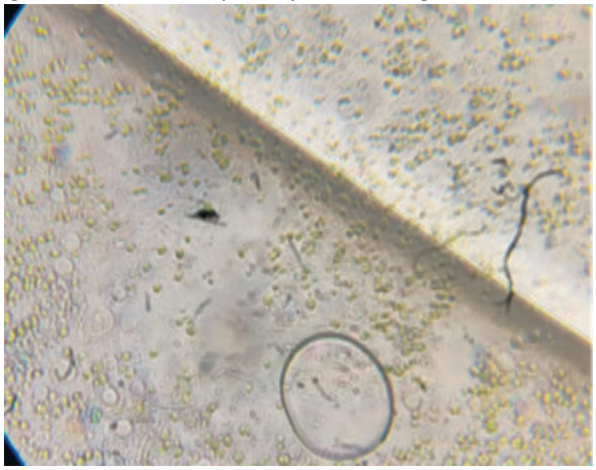

Figure: 1. Microscopic View Of Cyanobacteria

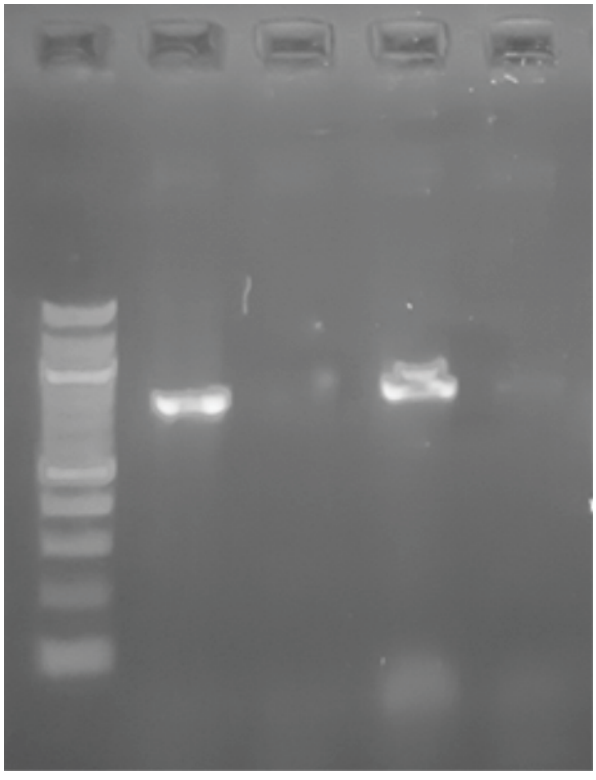

Figure: 2. PCR Amplified DNA. Lane 1 – 100bp Marker, Lane 2 – 800bp Amplicon Of 16s Rrna Gene, Lane – 3, Negative Control; Lane 4 – Positive Control

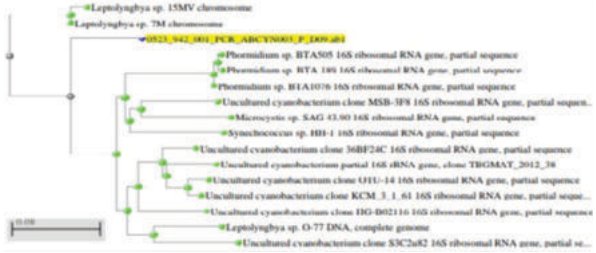

Figure 3: Top 15 Matches Of Cyanobacteria Make Phylogeny Tree.

DISCUSSION

The 16S rRNA sequence is highly conserved among bacteria, but it also contains regions of variability that can be used to differentiate between different species or even strains of bacteria. By comparing the 16S rRNA sequences of unknown bacteria to a database of known sequences, scientists can determine the identity and phylogenetic relationship of the microorganism. NCBI database revealed the best match with Synechococcus sp.HH1 with 79.61% of identity and E value 1e-136 (Fig). The best ten matches were analyzed for phylogenetic tree provide information which indicate the close match with Synechococcus sp. and other multicellular cyanobacteria too. Since, we use single cellular cyanobacteria which has best Hit with single cellular cyanobacteria Synechococcus sp.HH1 along with others close match Leptolyngbya sp 15MV chromosome, leptolyngbya sp . 7M chromosome , phormidium sp . BTA505 , uncultured cyanobacterium clone 36BF2, Unclured cyanobacterium clone MSB -3, uncultured cyanobacterium clone HG-B0, Phordium sp. BTA 189 , Leptolyngbya sp.O-77 DNA , Uncultured cyanobacterium clone OTU-1, (Fig 1.). Molecular identity of single cellular cyanobacteria is being clear as per the sequence match is Synechococcus sp.

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