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Metagenomic Analysis of Agricultural Soils to Identify Phosphate-Solubilizing Bacteria

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Abstract: ***Every living thing on Earth is significantly influenced by microorganisms, whether directly or indirectly. Many microorganisms are still unknown, and some of them are valuable and some are not. Whether intentionally or unintentionally, microbes have an influence on our environment and way of life. They are found across the whole planet, from the atmosphere to the deep oceans. Through active engagement in the carbon, nitrogen, sulphur, and phosphorus cycles, soil microorganisms contribute significantly to the maintenance of the natural ecological equilibrium. In the rhizosphere, phosphate-solubilizing bacteria (PSB) play a crucial role in facilitating the solubilization of inorganic phosphorus compounds into soluble forms that may be used by plants for nourishment. Agriculture industry is very interested in this variety of bacteria since they may be utilized as biofertilizers for crops. The identification of various and non-cultivable phosphate-solubilizing bacterial species is hampered by conventional culture-based approaches. In order to determine if phosphate-solubilizing bacteria are present in agricultural soils and whether they have any useful properties, metagenomic analysis is essential as it is a direct genetic examination of the genomes of microorganisms from an environmental sample. The diversity, structure, and function of microbial communities that are mostly uncultured and unknown can be discovered using this technique, which is culture independent. Phylogenetic links between microbes and new genes, enzymes, and biocatalysts can both be found by metagenomics investigation.***

Keywords: environment; microorganisms; agriculture soils; phosphorus; metagenomics; phosphate solubilizing bacteria; biofertilizers

1. Introduction

After nitrogen, phosphorus (P) is regarded as the most significant element in plant nutrition.[3] It functions as a crucial element in each of plants' primary metabolic processes, including photosynthesis, signal transduction, and respiration. However, the amount of soluble phosphorus in soil is often quite low (400–1260 mg kg1), necessitating the use of fertilizers in order to increase agricultural production and meet the rising demand brought on by the continued population expansion in the globe. Because of this, the overall demand for phosphorus fertilizers has been rising by 2.5–3.0% year. Despite the fact that phosphorus is abundant in the soil in both organic and inorganic forms, it is generally inaccessible to plants due to its complexation with metal ions.[2] In order to meet the need for phosphorus and increase agricultural productivity, agrochemicals have been used, which has resulted in a degraded ecosystem, unhealthy soil, and an unbalanced soil microbiota.[2] As a result, there is a need for a different, more economical, and environmentally acceptable method of biofortifying phosphorus. One such method is the use of phosphate-solubilizing microorganisms, which can solubilize insoluble phosphates in soil through a variety of mechanisms, including the secretion of organic acids, the production of enzymes, and the excretion of siderophores, which can chelate the metal ions and form complexes, making phosphates available for plant uptake. These microbes ensure plant growth and crop yield by promoting growth hormones like auxins, gibberellins, and cytokinins, antibiosis against pathogens, 1-aminocyclopropane-1-carboxylic acid deaminase, which enhances plant growth under stress conditions, improves plant resistance to heavy metal toxicity.[2] For metagenomic study, DNA is extracted from environmental samples, such as agricultural soils, which is then followed by high-throughput sequencing and bioinformatics analysis. This method sheds light on the microbial communities that exist in soil in terms of their taxonomic makeup, functional potential, and metabolic activities.[1] Metagenomic analysis has been an important method for the detection and characterization of PSB in agricultural soils in recent years. Researchers may examine the enormous genetic variety and functional potential of microbial communities related to phosphate solubilization by making use of next-generation sequencing technology. As we know that, through diverse phosphate solubilization methods, bacterial populations can encourage greater phosphorus (P) availability for plants and microorganisms in soil. The synthesis of extracellular phosphatases causes the breakdown of organic phosphate to liberate accessible phosphate. Understanding the diversity and richness of the bacterial population in soil, especially the bacteria that solubilize phosphate, may help us find solutions to the phosphorus scarcity that exists in soil ecosystems. The goal of this study is to conduct an in-depth review of the material that has been published by researchers using next-generation sequencing of the 16S rRNA gene, the diversity and relative abundance of bacterial phyla and genera were examined from six agricultural soil samples from Vietnam.[4] The alkaline phosphatase gene phoD abundance and the physico-chemical parameters were used to compare the phosphatase activity of each soil. Moreover, the researchers demonstrated the dominance of Firmicutes, Proteobacteria, Actinobacteria, and Chloroflexi.[4] The phyla Proteobacteria, Acidobacteria, Firmicutes, and Planctomycetes all showed positive correlations with total nitrogen. The abundance of various Proteobacteria genera exhibited a positive correlation with the phoD gene copy number.[4] While there was a negative correlation between Proteobacteria and sand concentration, the abundance of certain species showed a positive correlation with silt content. The results generated by the scientists clearly showed the impact of soil physico-chemical characteristics on the diversity of bacterial taxa, including those that could be engaged in phosphate solubilization.[4]

2. Metagenomic Sequencing

The study of genetic material directly extracted from environmental or clinical samples using a technique called sequencing is known as metagenomics. The process includes randomly sequencing samples' genomic DNA in an environment.[5] Metagenomics can provide a thorough understanding of how microbial communities interact and assist in locating specific species within microbial ecosystems.[6] Amplicon sequencing and shotgun sequencing are the two main sequencing methods used in metagenomics.[7] With the ultimate goal of obtaining a global understanding of how the microbial world functions, metagenomic represents a strategic concept that includes investigations at three major, interconnected levels, including sample processing, DNA sequencing, and functional analysis. Metagenomic has been separated into two strategies, functional based and sequence based, to extract the new biomolecules from the environmental samples. Steps of functional based analysis will require extracting DNA and cloning it into a vector. Transformation of a clone into an appropriate bacterial host cell, and d) screening for transformants. Finding clones that express a function is the powerful but difficult part of function-based approaches for metagenomic study. Success requires accurate transcription and translation of the gene or genes of interest, as well as the release of the gene product if the assay or screen calls for extracellular expression. The method's strength lies in the fact that it does not need that sequence analysis be able to identify the genes of interest, making it the sole method available to metagenomics, which has the capacity to discover whole new gene classes for either unknown or well-known activities. The major drawback is that most genes- possibly even all- will not be expressed in any given host bacteria used for cloning. Metagenome sequencing or sequence-based analysis can be used to identify the DNA sequences present across the whole metagenome. In this application, conserved sections of already-known genes or protein families are used to build DNA probes or primers. Only unique variations of recognized functional groups of proteins may be found in this approach.

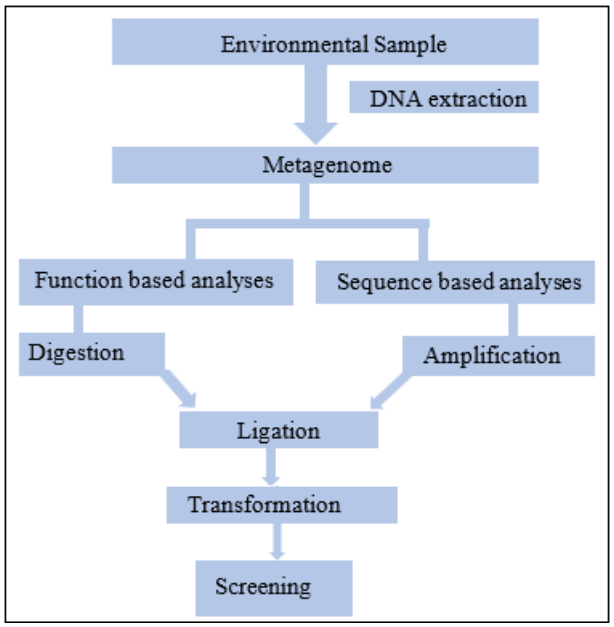

Figure 1: A diagram depicting the construction of libraries from environmental samples.

3. Metagenomic DNA extraction

The first stage in these metagenomic techniques is the isolation or extraction of environmental DNA. There are two ways to extract DNA; the first is direct DNA extraction, which involves lysing the cells directly.[8] There are several contaminants in environmental samples, such as humic chemicals, that must be removed from DNA in order to purify the DNA. Using electrophoreses, DNA may be purified on the basis of charge.[9] Extraction buffer [Tris-HCL (pH-8.0), 100mM sodium EDTA (pH 8.0), 1.5 M NaCl], and glass beads are added to the sample to extract DNA. The sample is then mixed for 2 minutes before being treated to sodium dodecyl sulphate and incubated for 1 hour at 65°C. Following incubation, the sample is centrifuged at 1400 rpm for 5 minutes. The lysate is then transferred to clean centrifuge tubes and isopropanol is added to it. After that, the tubes are held at 4°C for 2 hours. After centrifugation, the pellet is washed with 70% ethanol, air dried, and kept in Tris buffer.[10] Electrophoresis then confirms the presence of DNA. Then, either a molecular approach or a charge-based method of purifying DNA is used. While physical disruption techniques like glass beating or sonication can provide significant quantities of DNA, they frequently result in shearing, which is bad for the development of large insert metagenomic libraries. Therefore, bead beating can be replaced with enzymatic or chemical lyses. The second involves indirect DNA extraction, in this, samples are homogenized at pH 8.0 with 100mM Tris-HCl, 100mM SDS, and 0.1% CTAB present. The sample is subjected to centrifuge (100xg for 10 min) to eliminate contaminants. The amount of bacterial biomass that can be recovered through repeated mixing procedures is adequate for further DNA extraction. After many rounds of mixing, the supernatant is then collected and pooled. Finally, the microbial cells are separated and concentrated using a centrifuge running at 10,000 x g for 30 minutes. Utilizing microscopy, the effectiveness of bacterial recovery should be evaluated. After lysis, proteins and enzymes can be inactivated by exposing cells to proteinase. The indirect technique normally takes more time but is less contaminated. The approach produces less DNA because of limited target prokaryotic DNA, which reduces the yield but increases DNA size.[11] Comparing the two methods, it can be seen that the direct method (bead beading) produces a lot of nucleic acids, but the resulting nucleic acid extracts are frequently sheared and contaminated with humic acids. Additionally, tracts frequently contain unidentified amounts of extracellular and/or eukaryotic DNA. Although the indirect technique of extraction takes longer, it produced the DNA with the best purity and the least amount of DNA shearing. When aiming at bacterial communities, this technique is applied.[12]

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