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16S rRNA Gene Sequencing Technology For Microbes Identification

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16S rRNA gene sequencing has revolutionized the identification and classification of bacterial microorganisms by bypassing the constraints of traditional, time-consuming culture-based methods. By targeting the 16S rRNA gene—a highly conserved genetic marker present in all prokaryotes—this technology utilizes universal primers to amplify DNA while leveraging variable regions to differentiate between species. This method is particularly vital in agricultural microbiology for understanding soil fertility, nutrient cycling, and disease suppression. Although the process requires specialized technical expertise and equipment, its ability to identify unculturable bacteria and provide high-throughput data makes it an essential tool for modern environmental, medical, and sustainable agricultural research.

Keywords: 16S rRNA gene, microbial identification, bacterial sequencing, soil microbiome, PCR, bioinformatics, sustainable agriculture, microbial diversity.

Introduction

Microorganisms are present everywhere in nature, including soil, water, air, plants, animals and humans. In agriculture, microbes play an important role in soil fertility, nutrient cycling, plant growth promotion, and disease suppression. Accurate identification of microorganisms is essential for understanding their functions and applications in agricultural systems. Traditional methods based on morphology and biochemical characteristics are often time consuming and unable to identify many unculturable microorganisms. To overcome these limitations, molecular techniques such as 16S rRNA gene sequencing have become powerful tools for microbial identification.16S rRNA is a highly conserved genetic marker present in all bacterial and is widely used for microbial identification and evolutionary studies(Tringe & Hugenholtz, 2008).

What is 16S rRNA Gene Sequencing

16S rRNA gene sequencing is an amplicon based sequencing method that is used to identify and classify bacteria present in bulk and complex biological samples. The 16S rRNA was first introduced by Carl Woese and George.E. Fox in 1977. This method uses the highly conserved nature of the 16S ribosomal RNA (rRNA) gene present in all prokaryotes that also contains variable regions that differentiate between species. ribosomes are cellular structures involved in protein synthesis and are made up of proteins and ribosomal RNA (rRNA). In prokaryotes, ribosomes consist of 30S and 50S subunits that together form the 70S ribosome. The 30S subunit contains the 16S rRNA molecule. The 16S rRNA gene is about 1500 base pairs long and contains conserved and variable regions. Conserved regions are similar in most bacteria and help in gene amplification using universal primers, while variable regions differ among bacteria and help in their identification and classification.

Principles of 16S rRNA Gene Sequencing

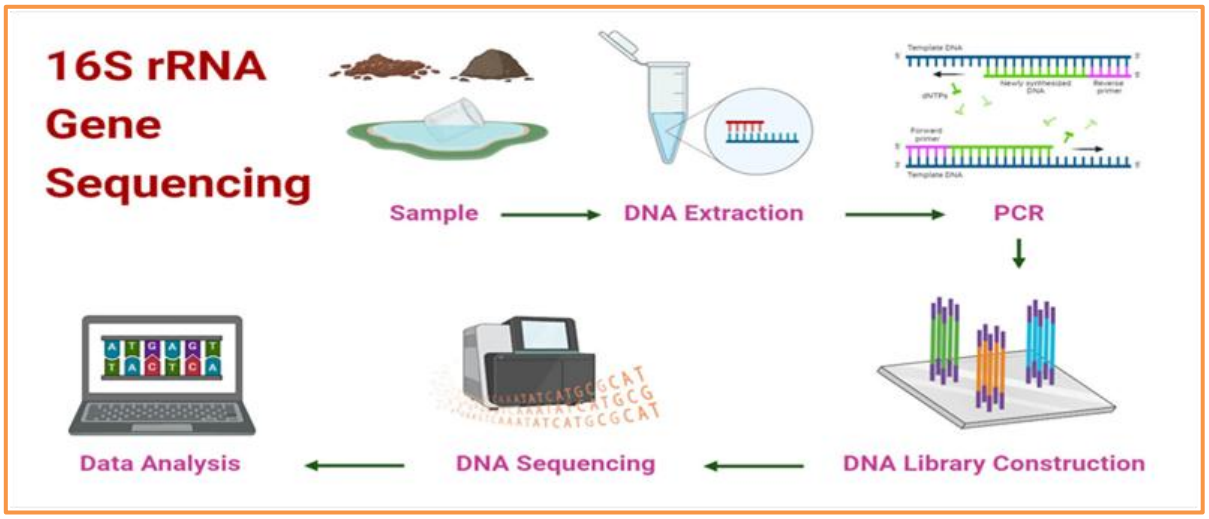
The principle of 16S rRNA gene sequencing is based on the analysis of the 16S ribosomal RNA gene present in all bacteria. This gene contains both conserved and variable regions. The conserved regions remain similar among different bacterial species, allowing the use of universal primers for amplification, while the variable regions differ between species and help in bacterial identification and classification. In this method, microbial DNA is first extracted from the sample and the 16S rRNA gene is amplified using Polymerase Chain Reaction (PCR). The amplified gene fragments are then sequenced to determine the nucleotide sequence. The obtained sequences are compared with reference databases to identify microorganisms and study their phylogenetic relationships. (Janda & Abbott, 2007)

Steps Involved in 16S rRNA Gene Sequencing

The process of 16S rRNA gene sequencing starts with the collection of samples from environments such as soil, water, plant roots, sediments, or other microbial habitats. After collection, microbial DNA is extracted carefully from the sample because good-quality DNA is essential for accurate sequencing results. Different extraction methods are used to break open microbial cells and isolate pure DNA from the sample.(Pichler et al., 2018)

1. Sample Collection

Samples containing microorganisms are collected from sources such as soil, water, compost, plant tissues, or rhizosphere soil. Proper sterile handling is important to avoid contamination and ensure accurate results.



2. Extraction of Microbial DNA

Microbial DNA is isolated from the collected sample by breaking open the cells and removing impurities. Good-quality DNA is essential for successful sequencing and accurate microbial identification.

3. PCR Amplification of 16S rRNA Gene

The extracted DNA is amplified using Polymerase Chain Reaction (PCR). Universal primers bind to the conserved regions of the 16S rRNA gene, allowing amplification of bacterial DNA. The variable regions help in identifying and differentiating bacterial species.

4. Purification of PCR Products

The amplified PCR products are purified to remove unwanted substances such as primers, enzymes, and impurities. This step improves sequencing quality and reduces errors during analysis.

5. Library Preparation

Sequencing adapters and barcode sequences are attached to the amplified DNA fragments. Barcodes help identify different samples when multiple samples are sequenced together.

6. **DNA Sequencing**

The prepared DNA libraries are loaded into sequencing platforms such as Illumina MiSeq or MiniSeq. The sequencing instrument determines the nucleotide order of the amplified 16S rRNA gene fragments.

7. **Bioinformatics and Data Analysis**

The obtained sequence data are processed using bioinformatics tools. Low-quality reads are removed, and the sequences are compared with databases such as SILVA, RDP, and NCBI GenBank to identify microorganisms and analyze microbial diversity.

8. **Interpretation of Results**

The final results help researchers understand the composition and diversity of microbial communities. In agricultural microbiology, this technique is useful for studying beneficial microbes involved in soil fertility, nutrient cycling, and plant growth promotion.

Advantages of 16S rRNA Gene Sequencing

This are the advantage of 16S rRNA Gene Sequencing as follow (Muhamad Rizal et al., 2020)

- Useful for studying microbial diversity, phylogeny, and community structure in different environments.
- Sequence data are stored in digital form, making sharing and comparison between laboratories easier.
- Supports rapid and streamlined workflows for microbial identification compared to many conventional methods.
- Widely used in environmental, agricultural, and clinical microbiology studies for detecting bacterial populations.

Limitations of 16S rRNA Gene Sequencing

This are the limitations of 16S rRNA Gene Sequencing as follow (Muhamad Rizal et al., 2020)

- Requires specialized instruments, technical expertise, Sequencing and laboratory setup can be expensive and bioinformatics knowledge for data analysis.
- Closely related bacterial species may sometimes show very similar 16S rRNA sequences, making precise identification difficult.
- The technique mainly identifies bacteria and provides limited information about microbial function or antibiotic resistance.
- Large amounts of sequencing data require careful processing and quality control during analysis

Future Prospects

The future of 16S rRNA gene sequencing is becoming more promising with the rapid development of sequencing technologies and bioinformatics tools. Modern sequencing methods are now faster, more accurate, and less expensive than before. These improvements make microbial identification easier and allow researchers to study large microbial communities in detail. In agriculture, 16S rRNA sequencing can help in understanding soil microorganisms, improving soil fertility, developing biofertilizers, and managing plant diseases. The technique is also expected to support sustainable farming and environmental monitoring in the coming years.

Conclusion

16S rRNA gene sequencing is an important molecular tool used for the identification and classification of bacteria. The presence of conserved and variable regions in the gene helps scientists differentiate microorganisms and study their evolutionary relationships. Compared to traditional culture-based methods, this technique provides faster and more accurate results, including the identification of unculturable bacteria. Although the method requires specialized equipment and data analysis, it has greatly improved the study of microbial

diversity and has become widely useful in agricultural microbiology, environmental science, and medical research.

References

1. Janda, J. M., & Abbott, S. L. (2007). 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/JCM.01228-07
2. Muhamad Rizal, N. S., Neoh, H., Ramli, R., A/LK Periyasamy, P. R., Hanafiah, A., Abdul Samat, M. N., Tan, T. L., Wong, K. K., Nathan, S., & Chieng, S. (2020). Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: Perspectives from a middle-income country. *Diagnostics*, *10*(10), 816.
3. Pichler, M., Coskun, Ö. K., Ortega-Arbulú, A., Conci, N., Wörheide, G., Vargas, S., & Orsi, W. D. (2018). A 16S rRNA gene sequencing and analysis protocol for the Illumina MiniSeq platform. *MicrobiologyOpen*, *7*(6), e00611. https://doi.org/10.1002/mbo3.611
4. Tringe, S. G., & Hugenholtz, P. (2008). A renaissance for the pioneering 16S rRNA gene. *Current Opinion in Microbiology*, *11*(5), 442–446.