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"OMICS" DRIVEN INSIGHT TO SOIL MICROBIOME: THE FUTURE OF SUSTAINABLE AGRICULTURE

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Microorganisms play a pivotal role in the agriculture production system by inducing several nutrient cycling systems, pest and disease dynamics, and soil quality. In the last several decades, these potentials of microbes are continuously getting explored for even higher production and at the same time maintaining the soil and environmental health. Careful alteration of the microbial community can significantly improve the quantity and quality of production. The modern cutting-edge research revolves around the better understanding of microbes, their interaction with the environment, association with root zone, utilization of secondary metabolites and many more aspects. In recent times, comprehensive research has led to the production of several biofertilizers, which has significantly improved the soil health and crop yield, especially in challenged areas demonstrated at the controlled environment and field application. However, soil microbiota's enormous complexity and diversity have possessed immense challenges in this field. The major problem in this research area is that approximately only 1% of the total microbes can be cultured *in vitro* (in the lab), while the vast majority is still unculturable. Over the year, an alternative approach has been developed that is culture-independent and based on isolation identification and quantification of genomes isolated from the target soil. In the meantime, gene sequencing technologies has been astonishingly improved, resulting much faster and cheaper sequence of genomes. Simultaneous sequencing or next-generation sequencing has transformed the human capacity to better understand the genome and classify the microbes taxonomically and functionally, which has opened a way of better agriculture management tomorrow.

Nuts And Bolts of Soil Microbiome from Metagenomic Insight

Soil is immensely diverse based on its physicochemical and biological properties. The diversity is due to the inherent parent material or a complex interaction of mineralogy,

ecology, environmental, and anthropogenic factors. The soil itself is an autonomous system that wharves several elementary processes, including nutrient cycling, different moisture regimes and microbial actions. The various ecosystem services are primarily dependent on microbes. However, as mentioned above, the study on such soil microbes was minimal because in vitro culture was possible. The recent development of two "omics" tools, i.e., metagenomics and metatranscriptomics in system biology, has laid the foundation for more in-depth study. This has been further improved due to the improvement of bioinformatics tools, more powerful computing ability and incorporation of high-level programming languages like python.

16s rRNA Gene: The Heart of the Process

The 16S rRNA has been proved to be a brilliant genetic marker for bacterial taxonomy due to its higher conservation nature in the bacterial and archaea domain. The 16S rRNA gene is the DNA sequence corresponding to rRNA encoding bacteria, which exists in the genome of all bacteria. The 16s rRNA gene has nine hypervariable regions ranging from V1 to V9. The conserved region has a unique benefit, to be used as an anchoring region for amplifying the variable region. Meanwhile, the variable area provides the essential element later used as a taxonomic marker. The number of copies of 16S rRNA is currently also used as an index of microbial lite history; higher numbers translate to favourable conditions and rapid multiplication. The copiotroph organisms have a higher number of operon copies, while oligotrophs have a lower number of operons.



Fig 1: Picture of Illumina HiSeq 2500 system, which is used in metagenomic sequencing

A composite soil sample contains hundreds or even thousands of bacterial species in complex communities. The majority of them are not culturable. A single unit constitutes such a community known as a "**metagenome**". Once again, the study of a particular constituent like a single gene or operon or another component is called "**targeted metagenomics**".

The 16S rRNA gene of the bacteria can be amplified using universal primers using PCR protocols. Subsequently, the sequence is later used as a biomarker to identify the taxonomic position of the microbe.

Among several regions, an extensive number of the literature suggests that the V3 and V4 region of 16s rRNA is the richest fragment for differentiating a large number of bacteria. As a result, the most popular high throughput sequencing platform company "Illumina" has adopted this region as a standard.

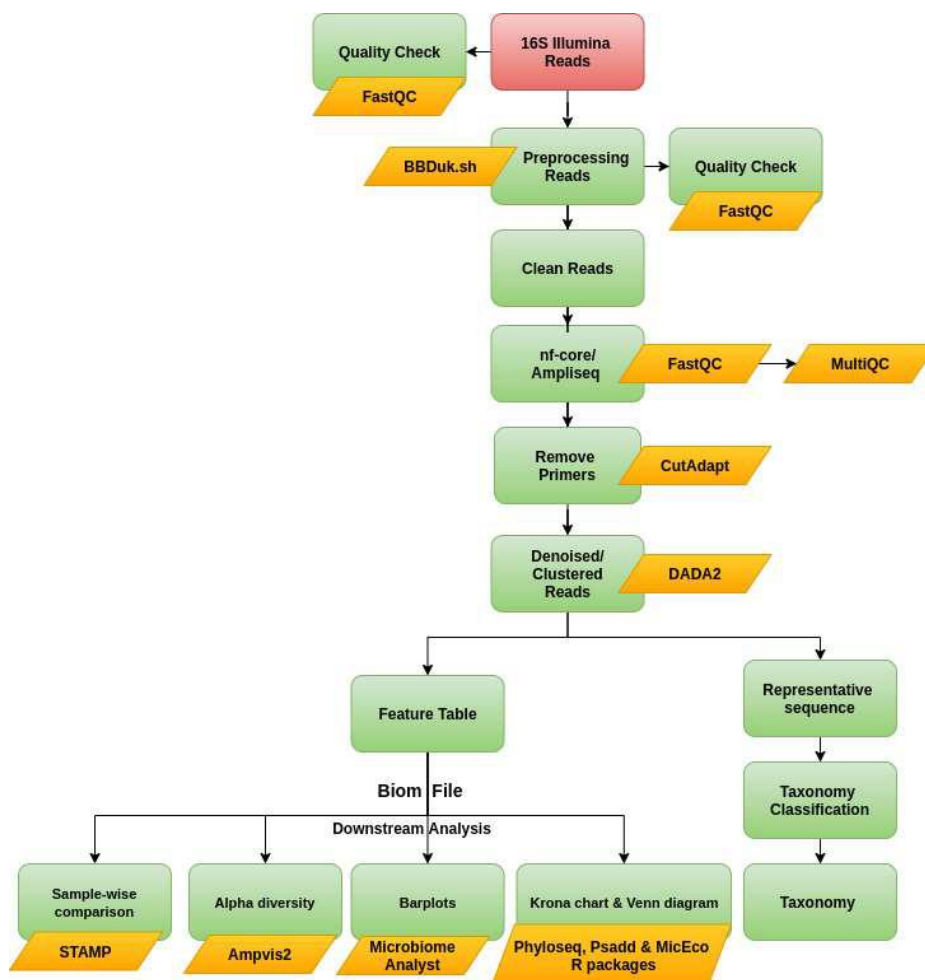


Fig 2: Exemplary workflow of 16s rRNA sequencing (V4 region) followed by identification, taxonomic classification and diversity analysis of bacterial community structure compost sample. (Source and copyright: authors of this article, agronomy section, ICAR-NDRI, Karnal)

Microbe Derived Biological Process and Its Impact on Modern Agronomy: How Can The Omics Help?

Since the last decade, bioinformatics tools have evolved so that 16S rRNA is not only used to identify the microbe. Instead, it can be used to assess several metabolic and biochemical processes. This phylogeny-driven metabolic prediction data is known as metabolic inference or functional potential.

The process can be understood from an angle of soil-borne pathogenic bacteria. An antibiotic is needed to suppress the bacterial disease in the plant. However, functional metagenomics can be used to determine the chances of antibiotic resistance. A more targeted and efficient antibiotic/strategy can also be used to be environmentally friendly. The procedure will involve the identification of specific bacteria as well as antibiotic-resistant gene, their mechanism of action and the mechanism of disease infestation in the crop. Nonetheless, highly specialized tools and mechanisms will be required to deploy such a methodology.

From the plant nutrition point of view, different nutrient cycle and their association with solubilizing and fixing bacteria plays the most crucial role. Functional metagenomics will bring up the whole process to the genomic level, which will help modify the system at the micro-level with higher precision and not break the agro-ecosystem.

In modern agriculture, it is scientifically proven that adding organic matter via incorporating different compost is essential to achieve better soil physical, chemical and biological properties and achieve sustainability. The composting itself is a primarily microbe driven process. The composting process varies significantly based on source material, temperature, resource, and quantity. Omics, especially metagenomics, can help identify different compost friendly bacteria such as cellulose degrading bacteria and the class and type of bacteria that can survive under a particular temperature regime. Once the compost is applied to the soil, the omics tool is the most efficient way to assess if the existing microbial community is getting succeeded by the microbes that came with the compost itself. As a result, the synergism, antagonism and commensalism among microbes and plants will also be more clearly visible.

Conclusion

Despite such enormous advantages and scope of application of omics tools such as metagenomics and transcriptomics in agronomy; the research has yet to pick the momentum. The technology is a very recent one, and due to its sophisticated workflow, agronomists worldwide are taking their own time to replace conventional analytical procedures with molecular works. Moreover, the sequencing setup used for high throughput sequencing such as Illumina high seq is also costly and requires a highly skilled workforce. However, in the coming days, it is foreseeable that omics tools will replace the conventional methods for exploring the vast world of soil microbiology and its interaction with agronomic practices.

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