Use of microbes in next generation biofertilizers

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Introduction

Biofertilizers are essentially microbes, composed of bacteria, fungi and blue-green algae that facilitate availability of nutrients for plant growth (Figure 01). Although biofertilizers have been used for a very long time, they have had very little success in replacing chemical fertilizers due to many challenges. However, with the ever-growing concerns of environmental pollution, negative effect on beneficial organisms and their involvement in human and animal health issues, has prompted to look for more improved and efficient biofertilizers that can reduce the usage of chemical fertilizers. Hence, the challenges facing the next generation biofertilizers could be listed as follows:-

1.Improving the efficiency of microbial inoculants such as plant growth promoting rhizobacteria (PGPR) , and plant growth promoting fungi (PGPF).
2.Use of microbial consortia to provide multiple benefits.
3.Improving stability of inoculants during transport and storage.
4.Formulation for maximum survival.
5.Providing a stable environment for improved efficiency.
6.Use of uncommon inoculants such as extremophiles and micro algae, and the development of customized biofertilizers to suit the conditions of the fields and their geographical locations.
7.Identifying and popularizing other characters of biofertilizers in order to use them for bioremediation, improved plant physiology, and degradation of pesticides.

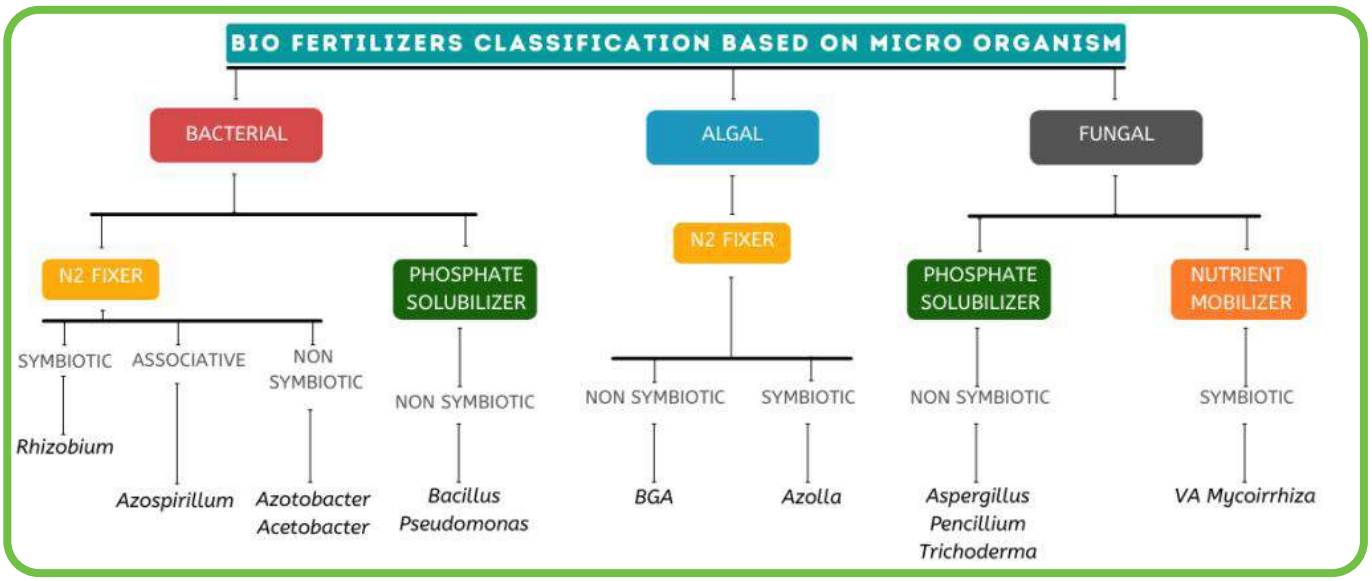

Figure 01: Biofertilizer classification based on microorganisms

With the rapid progress of technologies in molecular life sciences, these challenges can be supported by new integrated "omics" strategies, which include simultaneous use of metagenomics, metatranscriptomics, metaproteomics and metabolomics. Facilitated by ever increasing bioinformatic tools, the integrated omics has become the current tool in producing the next generation biofertilizers (Figure 02).

Use of Omics Tools in the Development of Efficient Biofertilizers

The emerging omics technologies which include Next Generation Sequencing (NGS), microarrays and other chip-based approaches have revolutionized the field of agriculture with the hope of converting conventional biofertilizers into precise, efficient, and reliable alternatives to chemical fertilizers. These technologies have concurred some of the usual challenges such as identification of mixed and unculturable strains, genetic variants, understanding complex pathways, and the signalling processes in inter species interactions. Tools of omics can play a role at several stages in the production of biofertilizer and these include;

1.Identification and selection of strains of the plant microbiome
2.Investigating the respective biochemical pathways
3.Improving the strains for more efficient performance
4.Genetic engineering of plants for better response

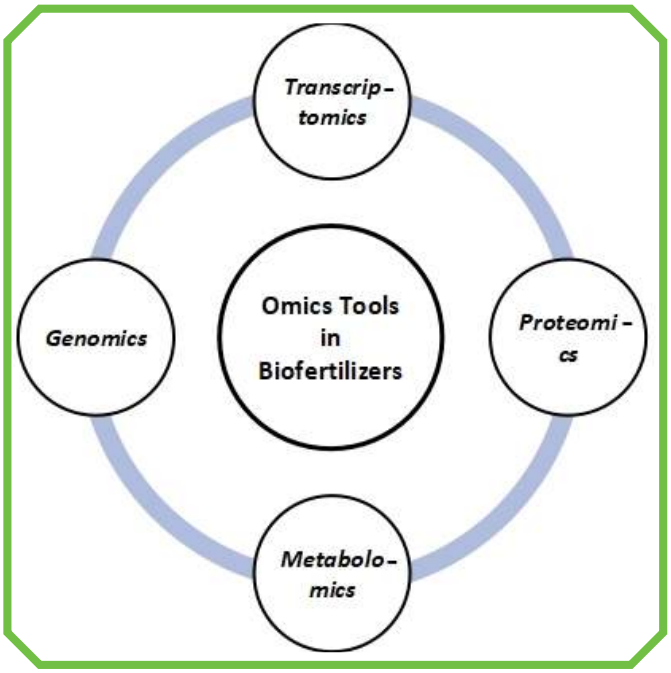

Figure 02: Different types of omics approaches for biofertilizers

Identification and Strain Selection for Biofertilizers

Whole Genome Sequencing (WGS) and RNA sequencing (RNA Seq), help to identify genes responsible and gene networks, including regulatory elements and metagenomics that facilitate massive amount of parallel sequencing. However, an in-depth understanding provided by specific genes, play an important role in culture selection for efficient biofertilizers. Therefore, some genomic strategies allow identifying of all the responsible microbes and the others select the best microbes for successful biofertilizers.

Metagenomics is the primary strategy for identifying all potential microbes in habitats that could expand the microbes in the biofertilizer industry. Inoculant strain development begins with the capture and identification of single strains or consortia (co-cultivated or co-inoculated microbial cultures). However, the success of these inoculants in commercially viable biofertilizers depends on their traits. These traits can be grouped into five levels;

1.Capture and refinement
2.Production
3.Establishment
4.Function,
5.Downstream impacts

Although the functional traits are important, establishment of microbes, which are more ecological, is equally important for efficient biofertilizer production. Henceforth, ideal microbial strains should possess both functional and ecological characters. The conventional culture-based capture methods may not pick up strains good at establishment and persistence. Moreover, many unculturable strains may possess ecological characters that make them superior in the environment as biofertilizers. Metagenomic tools of studying microbiomes include sequence-based metagenomics and functional metagenomics. The outputs of functional genomics contribute to strain selection and strain improvement, and improving plant response to microbes while the sequence-based metagenomics help to capture total strains in the rhizosphere. The combinations of these approaches can identify microbes for the next generation

biofertilizers, which are both functionally important and ecologically stable.

Sequence Based Metagenomics in the Capture of Potential Strains for Biofertilizers

The basic product of the sequence-based metagenomics approach in next generation biofertilizers, is the identification of microbial taxa in the rhizosphere samples to the genus or species level. Many parallel techniques have been developed under sequence-based metagenomics, which could be categorized into either targeted gene sequencing, or WGS. The prominent target gene sequence based metagenomic tool for bacteria is the 16S rDNA, because of the conserved sequences. For fungi, it is the Internal Transcribed Spacer (ITS) sequences.

Assigning taxa after a metagenomic analysis is done using a bioinformatic software and reference data base. For example, a metagenomic study conducted on soil microbial population and enzyme activities using MiSeq platform, used MiSeq Reporter for preliminary data analysis with Qiime, for assigning taxa at the species level based on Greengene database for bacteria and UNITE a web based database and sequence management environment for the molecular identification of fungi .

Screening of Potential Strains from Other Habitats

While it may be too early to think that cultures isolated from extreme niches could be developed into next generation biofertilizers, the fact that they may play a role in strain improvement either through genetic engineering or gene editing, cannot be ignored. Moreover, customized biofertilizers for geographic areas and temperature regimes may be a reality soon, where soils without ideal conditions for cultivation may become the norm. `Under these circumstances, soil reclamation may become the first step of microbial inoculant intervention. Such inoculants could also be used more as strains facilitating bioremediation rather than biofertilizers in contaminated soils. For instance, a rhizosphere metagenomic study conducted in oil-contaminated soil has revealed the significant presence of hydrocarbon degrading microbes.

Use of Extremophiles as a Prospective Source of Biofertilizer

Hyperthermophilic (extreme high temperature loving), thermophilic(high temperature loving), psychrophilic (cold loving), and halophilic (salinity loving) microbes could be considered as the extremophilic microbes. Conventionally, research on extremophiles have been dependent on culture-based techniques. However, with the introduction of metagenomics, this area of research has seen a massive expansion. A study on western deserts of Himalaya has identified many cold loving microbes with plant growth promoting (PGP) capabilities, under cold conditions. These capabilities include many properties ideal for next generation biofertilizers including; phosphate solubilization, aminocyclopropane -1- corboxy)ate (ACC) deaminase activity, production of molecules such as Indoleacetic acid (IAA), gibberellins, and production of siderophores. A metagenomic research on sediments of hypersaline Siberian soda lakes, identified microbes belonging to 45 phyla including 5 new species belonging to Candidate Phyla Radiation (CPR) and novel dominant members in previously identified groups of C, N and S cycling bacteria. Acidophiles (acid loving) and acid tolerant microbes are another source that may become beneficial in next generation biofertilizers, especially when some cultivable lands, such as rice fields have already become acidic. Cultures isolated from peat swamp forests of Southern Plant Growth Promotion (PGP). Thailand have shown several PGP traits such production of IAA, ALA, siderophores, phosphate solubilization and N_2 fixation at below 5pH range indicating the importance of this group in next generation biofertilizers.
Although targeted sequence metagenomics identify the potential microbes that could be used as inoculants for biofertilizers, it can only confirm the taxa involved. The true potential can only be investigated via the techniques of WGS, transcriptomics, proteomics, and metabolomics. While all these techniques could individually offer valuable information for selection of strains, it has become the new wave to use the integrated omics approach due to the ability of cross referencing between the technologies and the better prediction capabilities.

Use of Transcriptomics, Proteomics and Metabolomics in Microbial Strain Selection for Next Generation Biofertilizers

Although WGS can provide valuable information about

the genome composition, gene clusters and the functions of genes involved, further research at transcriptomic (mRNA), proteomic (protein) and metabolomic (metabolite) levels is required not just to confirm the predictions, but also to identify the expression levels, regulatory networks, and the metabolic profiles. Moreover, even when the relevant gene clusters could be identified by WGS, some microbial strains have not shown the expected PGP phenotypes. For instance, a research study on two plant- associated *Rhodopseudomonas palustris* strains (PS 3, and YSC 3) have indicated that only one strain was capable of PGP, even with very similar PGP gene clusters identified in both genomes. This proves the presence of the genes alone does not guarantee the functional roles of the genes. Besides, the gene expression for PGP microbes depend so much on the interaction with the relevant plant, maintained through chemical exudates released to the rhizosphere.

Metatranscriptomic approaches have become important to expand the basic information provided by metagenomics. Metatranscriptomics study the functional ecology rather than the annotated (predicted) ecology of the genomics. For instance, a study focusing on metagenomic and metatranscriptomic analysis of soil metagenomes clearly identified that although some strains are dominant in metagenomic analysis, functionally they may not be active.

Thus, metatranscriptomics provide information on how the expressions change temporally against changing environmental factors and interactions with other organisms. Hence, together with metaproteomic and metabolic profiling, meatranscriptomic studies could help establishment and persistence of microbial strains and consortia used in next generation biofertilizers.

Metaproteomics, which is the investigation of microbial proteins by mass spectrometry (MS) could be of two basic types, the intact protein MS/MS or "top down" and shotgun or "bottoms up" tandem MS/MS working with peptides. Developments in metaproteomics have identified metabolic pathways of microbes which can be used effectively to select efficient cultures for development of inoculants for biofertilizers. The workflow used in metaproteomic studies can have a direct impact on the outcomes. A seawater metaproteomic study that compared gel-based and gel free protein fractionation methods with four different protein databases, clearly showed that the number of proteins, taxonomic structures and functions of the proteins varied with the type of workflow used. Based on this evidence, the experimental workflows need to be diverse for better metaproteomic analysis.

Metabolomics involve metabolic profiling or metabolic fingerprinting. Many techniques can be used in metabolomics, including nuclear magnetic resonance (NMR), time of flight mass spectrometry (ToF-MS), Fourier-transform infrared spectrometry (FT-IR), GC MS, HPLC, and ultra-high-performance liquid chromatography (UPLC). While identifying the metabolite profile allows to recognize various PGP functional molecules such as growth factors and hormones, antibiotics, siderophores and many more, identifying these metabolites has also helped start a new trend in using metabolites in formulation with bioinoculants giving rise to more efficient next generation biofertilizers capable of surviving during transportation and storage.

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