Review Article

Molecular Characterization of Beneficial Soil Microorganisms: A Strategy for Enhancing CropProductivity

Abstract

The increase in global demand for food necessitates with increasing population has led to relay upon sustainable approaches to enhance crop productivity while maintaining environmental health. Beneficial soil microorganisms, such as *Rhizobacteria*, *Mycorrhizal* fungi, and *Actinomycetes*, play a pivotal role in improving soil fertility, promoting plant growth, and enhancing crop resilience to a biotic and biotic stressors. This review highlights various molecular strategies used for the characterization of these microorganisms, focusing on advanced techniques such as DNA based molecular identification, stable isotopes, arrays, integrated omics, etc. Additionally, it emphasizes the mechanisms by which beneficial microbes enhance crop productivity, including nitrogen fixation, phosphate solubilization, production of phytohormones, and the suppression of pathogens through biocontrol. The integration of beneficial soil microorganisms with precision agriculture and biofertilizer development is explored as a sustainable strategy to reduce chemical input and mitigate environmental challenges. This article provides a comprehensive understanding of molecular characterization techniques and their application in harnessing soil microbial diversity for enhancing crop yield, paving the way for sustainable agricultural practices.

Introduction

The rapid growth of the global population has led to a sharp increase in the demand for food production, posing a significant challenge to modern agriculture(Petrillo et al., 2021). Conventional agricultural practices, heavily reliant on chemical fertilizers and pesticides, have proven effective for short-term productivity but have also contributed to soil degradation, environmental pollution, and loss of biodiversity (Kour et al., 2020). These unsustainable practices threaten long-term food security, necessitating the adoption of eco-friendly and sustainable approaches to enhance crop productivity while preserving soil and environmental health (Timofeeva et al. 2023)."Soil is a region on the earth crust where geology and biology unite." Three interrelated factors i.e., physical fertility, chemical fertility and biological fertility compose soil fertility. Physical fertility refers to the physical properties of the soil i.e., structure, texture, porosity, and root fusion. Chemical fertility comprises nutrient status, pH, EC, base saturation of soil which is a decisive factor for a soil to be normal or problematic (acidity, alkalinity and salinity). Biological fertility includes the organisms that live and interact with the other components present in soil(De Souza et al., 2019). The soil organisms are highly dynamic and vary greatly in number but is least explored fertility factor(Bolton, 1993). Beneficial soil microorganisms, including rhizobacteria, mycorrhizal fungi, actinomycetes, archea, algae, insects, annelids, protozoa and other invertebrates have emerged as key players in sustainable agriculture(Glick, 2010). Among them microbial entities are very unique as microbial communities play very important role in plants' functions by influencing their physiology and development via directlyfacilitating plant growth through various mechanisms, such as nitrogen fixation, phosphate solubilization, production of phytohormones, and suppression of plant pathogens. By improving soil fertility and enhancing crop resilience to abiotic and biotic stresses, beneficial microorganisms not only boost agricultural productivity but also reduce the dependence on chemical inputs (Zhang et al., 2021). In 1904, L. Hiltner for the first time coined the term 'rhizosphere' to describe the highly dynamic region of soil in the proximity of plant roots in which microbiological activity is maximum(Hiltner, 1904). The roots secrete various organic and inorganic compounds such as referred as root exudates (Table 1) which help in colonization and survival of various microorganisms that in return encourage the plant growth known as plant growth promoting microorganisms (PGPM) (Nihorimbreet al. 2011). In- turn Plants secret out their photosynthetically fixed carbon into direct surroundings, i.e., spermosphere, phyllosphere, rhizosphere, and mycorrhizosphere, thereby nourishing the microbial community and contolling theiractivities (Nelson, 2004; Frey-Klett et al., 2007; Raaijmakers et al., 2009; Beredsenet al., 2012; Vorholt, 2012). In many regions, the healthy microbe population is still being threatened, and not at all promoted by agricultural practices(Agaras et al., 2015). The quantitative measurement of the increased microbial community as well as their activity is measured by the plating technique expressed as a 'rhizosphere effect' or Rhizosphere(Sukumaran et al., 2015). Recent advancements in molecular biology have provided powerful tools for characterizing and understanding the diversity, functionality, and mechanisms of beneficial soil microorganisms(Uzah et al., 2023). Techniques such as DNA based molecular identification, stable isotopes, arrays, integrated omics, etc. allow researchers to explore the vast microbial communities present in the soil(Franco-Duarte et al., 2019; Dabban et al., 2024; Streletskii et al., 2024). This review provides a comprehensive overview of the molecular characterization techniques and strategies employed to harness soil microbial diversity. It further explores their mechanisms in promoting crop productivity and discusses innovative approaches for optimizing their application in agricultural systems. By highlighting the role of beneficial microorganisms in enhancing crop yields, this review aims to provide a foundation for future research and practical implementation in sustainable agriculture.

Sugars	Glucose, fructose, sucrose, xylose, maltose, rhamnose, arabinose, raffinose				
Organic acids	Tartaric acid, oxalicacid, malic acid, acetic acid, citric acid, fumaric acid				
Amino acids	Leucine, valine, glutamine, asparagines, serine, glycine, glutamic acid, phenylalanine, threonine, tyrosine, lysine, proline, tryptophan				
Vitamins	Biotin, thiamine, pentothenate, niacin, choline, inositol, p-amino bezoic acid, pyridoxine, m-methyl nicotinic acid, 'M-factor'				
Nucleotide	Adenine, guanine, uridine, cytidine				
Flavanones	3,4-deoxy flavanones				
Enzymes	Invertase, amylase, protease, polygalacturonase, etc				

Table 1Microbially secreted compounds found in root exudates (Streletskii et al., 2024)

1. Mechanisms of Soil Microorganisms in Enhancing Crop Productivity

Plant growth promoting microorganisms can affect plant growth by different direct and indirect

mechanisms (Glick, 1995)(Fig 1).

1.1 Direct mechanisms

1.1.1Phytohormones production: The production of phyto-hormones is considered as one of the major mechanisms for the exclusive promotion of plant growth (Spaepenet al., 2007). Phytohormones are the signaling molecules acting as chemical messengers that in extremely low concentrations coordinate cellular activities of plants. The concentration of Auxin (Indole acetic acid) (Spaepenet al 2008), gibberellins (Bottini et al., 2004), cytokinins(timmusket al., 1999), jasmonic acid and brassinosteroids modulate plant growth and the response to stress(Masondo et al., 2024; Ali et al., 2024).

1.1.2 Indole acetic acid: Many important plants microbial interaction center on the formation of auxins (indole acetic acid as main plant auxins)(**Arora et al., 2024**). The IAA is responsible for the expansion, division and differentiation of plant cells, promotion of root and shoot elongation, apical dominance, etc(**Fan et al., 2024**).Generally, IAA released by rhizobacteria intervenes with various plant developmental processes because the endogenous reservoir of plant IAA may be altered by the acquisition of IAA that has been secreted by soil microbiota. IAA are produced by various rhizobacteria, e.g. *Azospirillum, Agrobacterium, Bacillus, Paenibacillus, Pseudomonas* and *Erwinia* (**Costacurta and Vanderleyden, 1995; Glick, 2012**).

1.1.3Gibberellin: Among all phytohormones, gibberellin producers are still not well understood (Pii*et al.*, 2015). Gibberellins (GAs) are a group of phytohormones associated with alterations in plant morphology, mainly in stem and root tissuesstem elongation, dormancy, flowering, germination and fruit ripening (Spence and Bais, 2015). *Acinetobacter calcoaceticus, Bacillus pumilus, Bacillus licheniformis*and *Promicromonospora*sp. are examples of bacteria that produce gibberellins and result in positive feedback in the endogenous GA of plants (Richardson *et al.* 2009; Figueiredo *et al.* 2010; Vacheron *et al.* 2013). *Bacillus siamensis* is able to produce GA and increases the growth of banana plants (Ambawade and Pathade 2015; Ahemad and Kibret, 2014). In *Azospirillum*, GA3 is the major GA type identified and appears to be involved in promoting plant growth (Cassanet al. 2009).

1.1.4 Cytokinins: The role of cytokinin is to promote cell growth and differentiation in plants(**Zhao et al., 2024**). They help the plant by the inhibition of aging of tissues and thus influence the leaf growth. The apical dominance induced by auxins is down-regulated by cytokinin's(**Sipes and Einset, 1983**). Cytokinins can be produced in soil by PGPR is an emerging alternative to enhance plant growth to improve yield and quality of crops(**Mughal et al., 2024**).

1.1.5 Ethylene and Abscisic acid: 1-aminocyclopropane-1-carboxylate (ACC) is a direct ethylene precursor exuded by roots. ACC is converted into α-ketobutyrate and ammonium by the enzyme ACC deaminase produced by PGPR(Hayat *et al.* 2010; Glick 2014). At low concentrations, ethylene mitigates detrimental effects of biotic and abiotic stresses (Glick 2014). Like ethylene, abscisic acid (ABA) also modulates plant development under stressful conditions (Glick 2015; Spence and Bais 2015). *Achromobacterxylosoxidans, Bacillus licheniformis* Pseudomonas putida are ABA producing bacteria that favourably influence plant homeostasis (Sgroyet al. 2009; Glick 2014).

1.1.6 Phosphate solubilization: Next to nitrogen, phosphorus is the second most important macronutrient required by the plants(Sen et al., 2024; Saber et al., 2005). The P content in average soil is about 0.05% (w/w) but because of its fixation and inadequate solubility in soil only 0.1% of the total P is available(Ducousso-Détrez et al., 2024). A diverse group of bacteria (Pseudomonads and Bacillus), Penicillium and Rhizoctonia fungal strains (Aspergilli, solani) yeasts (Yarrowialipolytica and Pichiafermentans), actinomycetes (Actinomyces Streptomyces), and cyanobacteria (Anabeanasp, Calothrix brauniiand Scytonema sp.) and arbuscular mycorrhizal fungi (Glomus fasciculatum) is recognized to be involved in microbial phosphate solubilization(Saha et al., 2024; Bai et al., 2024; Sharma et al, 2013).

1.1.7 Siderophore production: The production of **siderophores** is an alternative phenomenon of rhizobacteria(**Bisht et al., 2018**). These molecules have the efficiency to sequester Fe^{3+} ions, which are considered to play important role in metabolism and cell growth(**Ramos Cabrera et al., 2024**). In this way, the microbe that inhabit the plant roots compete for available iron present in the soil and may inhibit the proliferation of other pathogenic microorganisms in the rhizosphere (**Kumar et al. 2015**).

1.1.8 Biological nitrogen fixation: Nitrogen is required in the highest amount, and its availability is a major limitation factor for plant growth (**Courty***et al.* **2015**; **Bhattacharjee** *et al.* **2008**; **Figueiredo** *et al.*, **2013**). In a waterlogged rice field, the combination of *Anabaena* sp., a free-living diazotrophic nitrogen fixer bacterium, and *Azolla* compensate the shortage of nitrogen, hence, *Anabaena* may be referred to as a "biofertilizer" (**Bhuvaneshwari and Kumar 2013**; **Fosu-Mensah** *et al.* **2015**). In this

way, *Anabaena* contributes high nitrogen amounts (up to 50 kg ha⁻¹), reduces nitrogen loss via ammonia volatilization, and promotes plant growth (**Bhuvaneshwari and Kumar, 2013**).

1.2. Indirect mechanisms

1.2.1Biocontrols: PGPR can produce a wide variety of antimicrobial compounds for defense such as broad-spectrum antibiotics, lactic acid produced by *Lactobacillus lactis*, hydrolytic enzymes like lysozymes, chitinase, proteases, lipases and β ,1-3, glucanases that lyse the pathogenic microbes and numerous types of exotoxins and bacteriocins, which also have bactericidal activity (**Riley and Wetrz, 2002**).

1.2.2 Antimicrobial compounds: Antibiotics are heterogeneous group of organic, low molecular weight compounds that exert destructive effect on the growth or metabolic activities of other organisms. (**Duffy, 2003; Sharma et al., 2024; Kwon and Kim 2014**). On the other hand, bacteriocins differ from the traditional antibiotics in relatively narrow bactericidal spectrum i.e. toxic for only closely related to the producing strains (**Riley and Wetrz,2002**). Strains of *Leuconostocmesenteroides* subsp. *dextranicum* produce bacteriocins, but the compounds involved have not been completely purified and characterized yet (**Angelini, 2024; Chen et al., 2024**).

1.2.3 Stress tolerance: Within the major metabolic pathways studied and those involving the participation of rhizobacteria, stands out the metabolism involved in the production of phytohormones in the rhizosphere environment (**Araujo** *et al.* **2005**), and the induction of resistance to biotic and abiotic stresses (**Kang** *et al.* **2010**) has been reported. The accumulation of proline in plants acts as an osmoprotectant, maintaining the water potential under deficit conditions and facilitating water uptake from the soil (**Hanson** *et al.* **1979**).

1.2.4 Antioxidant enzymes

Plants produce a range of antioxidant enzymes such as catalases, peroxidases, and superoxide dismutases involved in scavenging free radicals (Simova-Stoilovaet al., 2008). The introduction of growth-promoting bacteria to the rhizosphere can greatly contribute to the production of antioxidant enzymes in plants(Araujo and Menezes, 2009; Kumar et al. 2009). Antioxidants can act directly in neutralizing the action of free radicals or can indirectly participate in enzymatic systems involved in this function (Moraes and Colla, 2006). The main antioxidant compounds with nutraceutical characteristics in plants are flavonoids, which act as potent antioxidants and metal chelators(Tapas et al., 2008).



Fig:1 Mechanisms of plant growth promotion by PGPR(Du, et al., 2020).

2. Identification and characterization of beneficial soil microbiota

The term "polyphasic taxonomy" was introduced by Colwell (1970) to refer to phenotypic, biochemical and molecular detection approaches. The first step towards identification of unknown microbe is to understand their phenotypic characters which include morphological characters, cultural characters and biochemical characters of microorganisms(**Bisht et al., 2019**).

2.1. Phenotypic characterization

The size, shape and arrangement of cells of microorganisms vary with species to which they belong(**Compant et al., 2024**). The size of some microbes is given in **Table 2**. Bacteria have three basic shapes or morphological type. Coccus, bacillusand spiral (**Fig 2**)(**Shreshtha et al., 2024**). Some multicellular microorganisms form the thin-walled, profusely branched filaments called hyphae(**Arellano-Wattenbarger et al., 2024;Banik et al., 2024; Fonseca-García et al., 2024**). Microorganisms are also recognized on the basis of their cultural characteristics which can provide clues to identify unknown microbes such as shape (circular, filamentous, rhizoid), size (small, medium, large), color, margin(entire, irregular, undulate, lobate), texture(soft, hard, mucoid), optical characters (transparent, opaque,), elevation (flat, convex, raised) and surface (smooth, rough, shiny)(**Sangeetha &Thangadurai, 2013; Woźniaket al., 2019**)

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Organisms	Significance	Size (µm)	Morphology
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Achromatiumoxaliferum	Sulfur chemolithotroph	35 x 95	Cocci
Beggiatoa species	Sulfur chemolithotroph	50 x 160	Filaments
Clostridium botulinum	Food poisoning	3-8	Rods
C. tetani	Tetanus	4-8 X 0.5	Rods
Corynebacterium diphtheriae	Diphtheria	3-6 x 0.6-0.8	Palisade
Escherichia coli	Prokaryotic model organism and indicator of fecal contamination	1x2	Rods
Epulopsciumfishelsoni	Most enignamotic group of gigantobacteria. 16s rRNA subunit is related to <i>Clostridium</i>	80 x 600	Rods with tapered end

Lyngbya majuscule	Produce altillatoxin and kaklitoxin, causal organism of seaweed dermatitis	8 x 80	Filaments
Magnetobacteriumbavari cum	Magnetotactic bacterium	2 x 10	Rods
Mycobacterium tuberculosis	Tuberculosis	0.5-4	Rods
Mycoplasma pneumonia	Pneumonia	0.2	Pleomorphic
Neisseria meningitides	Meningitis	1	Cocci
Pasturella pestis	Plague	1-2	Coccobacilli
Salmonella typhi	Typhoid	1-3 x 0.5-0.6	Rods
Staphylococcus sp	Boils	0.8	Cocci in clusters

Treponema pallidum	Syphilis	6-14	Spiral
Thiomargaritanamibiensi	Largest bacteria ever discovered	750	Cocci in chains
S			
Thiovulum majus	Sulfur oxidizing, fastest swimming bacteria with the speed of 615 µm/sec	18	Cocci



Figure 2 Morphological features of different microorganisms (AgSolutions; Australia; https://agsolutions.com.au/soil-microbes-sustainability/)

2.2Biochemical and physiological characterization

Biochemical characterization of microbes is based on the various enzymes or metabolic products produced by them such as carbon utilization test, citrate utilization test, Voges Proskauer test (Vedi and Pandey, 2024; Saeed et al., 2024). In Fatty acid methyl ester (FAME) analysis, fatty acids are extracted from the membranes of microorganisms, chemically altered to form volatile methyl esters, and analyzed by gas chromatography (GC)(Zhao et al., 2024). The resulting GC chromatogram is compared with reference chromatograms in a database containing data for thousands of bacterial isolates to identify the unknown microorganism (Fernandes, 2006; Zaratti et al., 2024; Bisht et al., 2019).

2.3. Molecular Characterization

2.3 DNA-Based techniques

The techniques include Restriction Fragment Length Polymorphism (RFLP), Amplified Ribosomal DNA Restriction Analysis (ARDRA) and Pulse Field Gel Electrophoresis (PFGE) (Fusco et al., 2023; William *et al.*, 1990).RFLP is preliminary genetic analysis that allows individual to be identified on the basis of their unique pattern of restriction enzyme cutting in specific regions of DNA(Dabban et al.,

2024). AFLP uses specific adaptors are ligated to enzyme restricted DNA which is subsequently amplified using primers from the adaptor and restriction site-specific sequences(Monteiro et al., 2024). ARDRA employs digestion of amplified ribosomal DNA with different restriction enzymes and a profile is obtained using the combination of these patterns(Kalia and Parshad, 2013; Maslow, 1993). PFGE uses in situ lysis of bacterial whole-cells in agarose plugs to release DNA which protect the chromosomal DNA from mechanical damages(Bashbosh et al., 2024; Dubey et al., 2024; Qurbani et al., 2024). 16S rRNA gene sequence is thus used for inferring the phylogenetic relationship among bacteria based on its universal distribution and highly conserved nature, (Hammoudeh et al., 2024; Botan et al., 2024). Thereafter, sequence comparing software packages such as BLAST and CLUSTAL X are used for alignment of 16S rRNA gene sequence. (Bisht et al., 2022; Li et al., 2024). Characterization of newly isolated strains by different molecular methods is summarized in Fig. 3.DNA-DNA hybridization is based on a comparison between whole genome of two bacterial species(Ansari et al., 2024; Pasquer et al., 2010).





2.3.2Other advanced molecular techniques

a. FISH (Fluorescence In Situ Hybridization)

To visualizeRNA and DNA targets, *in situ*—fluorescence (FISH) and chromogenic (CISH) detectionmethods are used (**Román-Lladó et al., 2024**). With the development of fluorescent labels radioactive labels were replaced by non-isotopic dyes(**Landegent***et al.*, **1984**; **Pinkel** *et al.*, **1986**; **Pinkel** *et al.*, **1988**). In 1989, DeLong first used fluorescently labeled oligonucleotides for the detection of single microbial cells(**Aggarwal et al., 2024**; **Kliot and Ghanim, 2016**; **Luongo et al., 2024**; **Tseng**

et al., 2024). Oligonucleotides were labeled with horseradish peroxidase (HRP) that used fluoresceintyramide as a substrate(Herr et al., 2015; Ye et al., 2014; Yan et al., 2022). The sensitivity of FISH in natural samples was significantly increased using polyribonucleotide probes labeled with several fluorochrome molecules (DeLong *et al.*, 1999;Vági, et al., 2014; Zimmer and Wähnert, 1986).

b. Microautoradiography

A radiotracer is used for incubation of sample to label microbial cell using radioisotopic compounds like soft beta emitters (³H, ¹⁴C, ³³P) or strong beta emitter like ³²P(**Rogers** *et al*, 2007). After incubation the samples are fixed in paraformaldehyde or ethanol and washed to remove surplus radiotracer(**Song**, *et al.*, 2024; **Wasmund et al.**, 2024; **Gougoulias et al.**, 2014;Carman, 2018). Silver grain clumps, so developed, can be easily seen clearly using transmission light microscopy/ bright-field microscopy/ phase contrast microscopy/ LSM (Laser Scanning Microscopy)(Ginige et al., 2005; Ouverney and Fuhrman, 1999).Fig. 4shows stepwise procedure for microautoradiography(Nielsen and Nielsen, 2005).



Figure 4: Step wise procedure of microautoradiography

c. Sequencing technology

Due to size limit of Sanger sequencing (i.e., 100 to 1000 base pairs), longer sequences are subdivided into smaller fragments that can be sequenced separately(**Muñoz-Ramírez et al., 2024; Siew et al., 2024; Li et al., 2024**).Sanger sequencing is still used for sequencing, because of its low rate of error, long read length (> 700 bp) and larger insert sizes (i.e. > 30 Kb)(**Chen et al, 2014; Sanger et al., 1977; Soodet al., 2008; Zaytsevetal., 2024**).Among the NGS technologies, the 454/Roche and the Illumina/Solexa systems can be applied to metagenomic samples(**Satam et al., 2023; Sharma et al., 2024**). The pyrosequencing method includes the sequential addition of all four deoxynucleoside triphosphates, which, if complementary to the template strand, are added by a DNA polymerase(**Nafea**)

et al., 2024; Mandlik et al., 2024).Illumina/Solexa technologyimmobilizes random DNA fragments on a surface and then performs solid surface PCR amplification, resulting in clusters of identical DNA fragments(Chettri, et al., 2024; Minocheet al., 2011; Meera Krishna et al., 2019; Raffan and Semple, 2011; Takeda et al., 2019; Bowman et al, 2015; Davis et al., 2013). The Applied Biosystems SOLiD sequencer has been extensively used, for example, in genome resequencing. This technology was developed by Life Technology in 2006(Chettri et al., 2024; Remesh et al., 2024; Ho *et al.*, 2011; Mandlik et al., 2024; Chettri et al., 2024). This procedure include—hybridization of primer, selective ligation of the probes, four-color imaging, and cleavage of probe—is repeated again and again, the number of cycles detect the eventual read length (Metzker, 2009).

d.Omics techniques:

Omics include an increasingly broad range of fields, which ranges from genomics (the quantitative study of protein coding genes, regulatory elements and noncoding sequences), transcriptomics (RNA and gene expression), proteomics (focusing on protein abundance), and metabolomics (metabolites and metabolic networks)(**Botta et al., 2024; Yow et al., 2022)**(Fig. 5).



Figure 5:Schematics representation of omics dogma describing the network the processing of DNA, RNA, proteins and metabolites and method used(Yow et al., 2022).

1. Genomics

Genomics is the study of an organism's genome, or genetic material. Genomics is an emerging area of molecular biology where novel techniques were employed in order to get an insight of the complex, biological function of the genome.

1.1 Metagenomics

Metagenomics is the culture-independent technique to analyze complete genome of microbial communities present in a particular environment sample(Ejaz et al., 2024; Yu et al, 2024).Phenolics and humic acid interfere with the downstreaming applications such as polymerase chain reaction (PCR) amplification and cloning of genes (metagenomic library construction)(Debnath *et al* 2010; Devi et al., 2024).The idea of cloningDNA directly from environmental samples was first proposedby Pace (Pace *et al.*, 1985; Schmidt *et al.*, 1991; Healy *et al.*, 1995). DeLong's group reported libraries constructedfrom prokaryotes from seawater (Stein *et al.*, 1996; Suwannaphan et al., 2024).

1.2 Transcriptomics:

Transcriptomics is the overall scanning of the approx. 50,000 known genes that are transcribed into mRNA from the three-billion-letter human genome(Aslam et al., 2024). The advantage of this new technique is that instead of examining one gene at a time, it analyses the complete transcriptome in one single go, which is the full set of all mRNA molecules present at a particular time in a defined cell population(John Martin et al., 2024; Vohra et al., 2024). All genes and most proteins can be considered as instruments to make the biochemical composition and there by the physiological identity of an organism(Raza et al., 2024).

1.3 Proteomics

Proteins are the most important components of major biochemical and signaling pathways, studies at protein levels are crucial to provide insight on molecular mechanisms underlying growth, development, and their interactions with the environment(**Pan et al., 2024; Macaulay** *et al.,* **2005**). The genome contains all of an organism's genes, having the codes for the proteins require for the function. Proteomics is the comprehensive investigation of proteins and their biological functions and processes(**Kaya, 2024; Siddiqui and Saeed, 2024**).Once the sequence of an organism's genome is obtained, it can be compared to that of other organisms to find genes that are similar to those already known(**Cataldo et al., 2024; Shiaw-Lin** *et al.,* **2005; Tasleem et al., 2024**).

1.4 Metabolomics(metabonomics)

It is the integrated study of the metabolome, the entire set of small metabolites (e.g. hormones, metabolic intermediates, signalling molecules, and secondary metabolites) of biological sample (Steuer *et al*, 2003). The metabolic state reflects the encoded product of genome which is being modified by environmental factors (Kaddurah-Daouk, 2006). NMR and other spectroscopic methods such as MS, GC/MS can be used to generate metabolic profiles(German *et al.*, 2005; Fiehn, 2002; Kristal, and Shurubor, 2005).In MALDI-TOF (matrix assisted laser desorption ionization) version of mass

spectrometry, the sample is ionized and vaporized by a laser(Chen et al., 2023). The time of flight (TOF) for each ion depends on its mass/charge ratio- the smaller this ratio(Bielen et al., 2024; Dash et al., 2024; Fontana et al., 2012)(Fig 6).



Figure 6:Integrating omics technology:genomics, proteomic, transcriptomic, and metabolomic(Fontana et al., 2012)

e. Arrays

DNA microarrays are a firmly rooted method for determining gene expression levels(Cummings and Relman, 2000).

A. Oligonucleotide Microarrays

Oligonucleotide microarrays have emerged as a preferred platform for genomic analysis beyond simple gene expression profiling(**Chung** *et al.*, 2009). Each chip may contain over 6 million features, each feature comprising millions of copies of a distinct probe sequence (**Gregory** *et al.*, 2008; **Mockler and Ecker**, 2005). The hybridization events can be detected, owing to the labelling of the bound complementary target, by high resolution scanning or imaging(**Wong and Medrano**, 2005).

B. cDNA Microarrays

cDNA microarrays are designed by mechanically attaching probes (e.g., amplified PCR products, oligonucleotides, or cloned DNA fragments) onto a solid substrate made up of glass having desired physico-chemical properties (e.g., excellent mechanical stability, durability and chemical resistance) (Gregory *et al.*, 2008; Mockler and Ecker, 2005; Zhou and Thompson, 2002). The disadvantages

related with oligonucleotide arrays include the associated costs and low sensitivity due to short sequences used in this technology (Schulze and Downward, 2001).

3. Applications in Agriculture

3.1 Beneficial microbe based biofertilizers

The major limitation of crop yield in developing nations globally, and particularly among resource-poor farmers, is soil infertility(Vlek and Vielhauer, 1994; Chen, 2006; Garcha and Samredhi, 2024). The term biofertilizer (microbial inoculants) can be defined as a preparation having live or latent cells of active microbial strains capable of nitrogen fixing, phosphate solubilizing which can be used for application of seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant(NIIR Board, 2004). The formulation of inocula, method of application and storage of the product are all critical to the success of a biological product. In general, there are 6 major steps in making biofertilizer(Bagga et al., 2024). First of all, active organisms must be decided (Santos et al., 2024). Then, isolation is made to separate target microbes from their habitation. Usually, organism is isolated from plants root or by luring it using decoy such as putting cool rice underground of bamboo plants(Fadiji et al., 2024; Kumar et al., 2024). At the end, biofertilizer should be tested on large scale to determine the effects and limits at different environment. Usually, biofertilizers are being prepared as carrier-based inoculants comprising active microorganisms(Schenk et al., 2024; Miranda, et al., 2024) (Fig. 7). The properties of a good carrier material for seed inoculation are inexpensive and available in adequate amounts(Zhao et al., 2024).



Figure 7: Commercial biofertilizers manufacturing process(Bagga et al., 2024)

3.2Integration with Precision Agriculture

Commonly used microorganisms as biofertilizers are nitrogen fixers, potassium solubilizer and phosphorus solubilizer, or combination of molds or fungi. These bacteria have close relationship with plant roots such as *Rhizobium* has symbiotic association with legume roots, and Rhizobacteria colonize on the surface of root or in rhizosphere soil(**Asad and Ahmed 2024**). The phospho-microorganism mainly bacteria and fungi make insoluble phosphorus available to the plants(**El-Moustaqim et al., 2024**). While Rhizobium, Blue Green Algae (BGA) and Azolla are crop specific, bio-inoculants like *Azotobacter, Azospirillum*, Phosphorus Solubilizing Bacteria (PSB), and Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad spectrum biofertilizers. VAM is fungi that are found associated with majority of agriculture crops and enhanced accumulation of plant nutrients (**Gupta &Samnotra, 2004; Gupta, 2004**). Examples of free-living nitrogen fixing bacteria are obligate anaerobes (*Clostridium pasteurianum*), obligate aerobes (*Azotobacter*), facultative anaerobes, photosynthetic bacteria (*Rhodobacter*), cyanobacteria and some methanogens(**Rahim, 2002**).

4. Future Perspectives

There are several views about biofertilizers. It is often perceived to be more expensive than the chemical fertilizers due to the lack of skills and technology to produce biofertilizer products from abundant wastes. Besides, the effect on the crops is slow, compared to chemical fertilizers. Special care such as storage or mixing with powders is also needed to handle microbial inoculum to remain effective for extended use. As biofertilizers contain living microbes, their effectiveness therefore depends on environmental conditions. Hence, outcomes are bound to be inconsistent. Short shelf life, lack of suitable carrier materials, susceptibility to high temperature, problems in transportation and storage are biofertilizer bottlenecks that still need to be solved in order to obtain effective inoculation.

Conclusion

Beneficial soil microorganisms are essential components of sustainable agriculture, offering eco-friendly solutions to enhance crop productivity while minimizing environmental impacts. By improving soil fertility, promoting plant growth, and providing resilience against various stresses, these microorganisms serve as natural allies in addressing the challenges of modern agriculture. The molecular characterization of soil microbial communities using advanced techniques such as DNA based molecular identification, stable isotopes, arrays, integrated omics, etc. has significantly expanded our understanding of their diversity and functional roles. Integrating these microorganisms into agricultural practices through biofertilizers, biostimulants, and precision farming not only reduces dependence on chemical inputs but

also contributes to long-term soil health and environmental sustainability. Despite the advancements, challenges such as scalability, strain-specific adaptability, and knowledge gaps in complex microbial interactions remain. Future research should focus on overcoming these challenges, fostering innovations in microbial engineering, and expanding practical applications to ensure global food security. By harnessing the potential of beneficial soil microorganisms through molecular and biotechnological approaches, we can pave the way for a more sustainable, productive, and environmentally conscious agricultural system. This review underscores the critical role of these microorganisms in shaping the future of agriculture and highlights the need for continuous research to unlock their full potential.

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