Review Article

Beneficial Soil Microorganisms in Enhancing Crop Productivity: A Review

Abstract

As the world's population grows, there is a greater need for food, which has made sustainable methods necessary to boost agricultural yields while preserving the environment. Rhizobacteria, mycorrhizal fungi, and actinomycetes are examples of beneficial soil microorganisms that are essential for increasing soil fertility, encouraging plant development, and strengthening crop resistance to biotic and abiotic stresses. The several molecular approaches utilized to characterize these microbes are highlighted in this study, with an emphasis on cutting-edge methods including arrays, stable isotopes, DNA-based molecular identification, integrated omics, etc.It also highlights the ways in which beneficial bacteria increase agricultural yield, such as through phosphate solubilization, nitrogen fixation, phytohormone synthesis, and disease reduction via biocontrol. One sustainable way to lower chemical input and lessen environmental issues is to use beneficial soil microorganisms into precision farming and the creation of biofertilizer. This article offers a thorough grasp of molecular characterisation methods and how to use them to increase crop output by using soil microbial diversity, opening the door to sustainable farming methods.

Key Words: Rhizobacteria, mycorrhizalfungi, actinomycetes, biocontrol and biofertilizer

Introduction

Modern agriculture faces a major problem as a result of the high rise in the need for food production brought on by the world's population expansion (Petrillo et al., 2021). Although traditional agricultural practices, which mostly rely on chemical fertilizers and pesticides, have been successful in increasing short-term production, they have also led to biodiversity loss, environmental pollution, and soil deterioration (Kour et al., 2020). In order to increase agricultural output while maintaining soil and environmental health, eco-friendly and sustainable methods must be adopted, as these unsustainable practices endanger long-term food security (Timofeeva et al., 2023"Soil is an area of the earth's crust where biology and geology come together." Soil fertility is made up of three interconnected components: physical fertility, chemical fertility, and biological fertility. The term "physical fertility" describes the soil's physical characteristics, such as its structure, texture, porosity, and root fusion. Nutrient status, pH, EC, and base saturation—which determines whether a soil is normal or problematic—are all components of chemical fertility (acidity, alkalinity, and salinity). The organisms that coexist and interact with the other elements found in soil are considered biologically fertile (De Souza et al., 2019). Although the quantity of soil organisms varies widely and they are extremely dynamic, the fertility component is the one that is least studied (Bolton, 1993).Rhizobacteria, mycorrhizal fungi, actinomycetes, archea, algae, insects, annelids, protozoa, and other invertebrates are examples of beneficial soil microorganisms that have become important components of sustainable agriculture (Glick, 2010).Microbial communities are particularly special among them since they have a significant impact on the physiology and development of plants by directly promoting plant growth through a variety of processes, including nitrogen fixation, phosphate solubilization, phytohormone

synthesis, and plant pathogen suppression.Beneficial microorganisms increase agricultural output and lessen reliance on chemical inputs by increasing crop tolerance to biotic and abiotic challenges and boosting soil fertility (Zhang et al., 2021).The word "rhizosphere" was originally used in 1904 by L. Hiltner to refer to the extremely dynamic area of soil near plant roots where microbiological activity is at its highest (Hiltner, 1904). Known as plant growth promoting microorganisms (PGPM), the roots release a variety of organic and inorganic substances, such as root exudates (Table 1), which aid in the colonization and survival of different microorganisms that in turn promote plant development (Nihorimbreet al. 2011).In- turn Plants secret out their photosynthetically fixed carbon into the rhizosphere, mycorrhizosphere, phyllosphere, and spermosphere, which feeds the microbial population and regulates its activities. **(Nelson, 2004; Frey-Klett*et al*., 2007; Raaijmakers *et al*., 2009; Beredsen*et al*., 2012; Vorholt, 2012)**.The population of beneficial microorganisms is still in danger in many areas, and agricultural methods do not in any way support them (Agaras et al., 2015).The plating approach, which is referred to as the "rhizosphere effect" or Rhizosphere, quantifies the increased microbial community and its activity (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,integratedomics, 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.

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

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

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

1.1.1 Production of phytohormones: One of the main mechanisms for the exclusive encouragement of plant development is thought to be the synthesis of phytohormones (Spaepenet al., 2007). At very low concentrations, phytohormones—signaling chemicals that function as chemical messengers—coordinate plant cellular activity. Plant development and stress response are influenced by the concentrations of gibberellins (Bottini et al., 2004), cytokinins (Timmusket al., 1999), auxin (Indole acetic acid) (Spaepenet al., 2008), jasmonic acid, and brassinosteroids (Masondo et al., 2024; Ali et al., 2024).

1.1.2 Indole acetic acid: The production of auxins—of which indole acetic acid is the primary plant auxin—is at the heart of several significant plant-microbe interactions (Arora et al., 2024). According to Fan et al. (2024), the IAA is in charge of the growth, division, and differentiation of plant cells as well as the encouragement of root and shoot elongation and apical dominance.Since the acquisition of IAA produced by soil microbiota may change the endogenous reservoir of plant IAA, IAA released by rhizobacteria often interferes with a variety of plant developmental processes. Azospirillum, Agrobacterium, Bacillus, Paenibacillus, Pseudomonas, and Erwinia are among the rhizobacteria that generate IAA (Costacurta&Vanderleyden, 1995; Glick, 2012).

1.1.3Gibberellin: Gibberellin makers remain poorly understood among all phytohormones (Piiet al., 2015). According to Spence and Bais (2015), gibberellins (GAs) are a class of phytohormones linked to changes in plant morphology, mostly in stem and root tissues, including stem elongation, dormancy, blooming, germination, and fruit ripening. Bacteria that generate gibberellins and cause positive feedback in the endogenous GA of plants include Acinetobacter calcoaceticus, Bacillus pumilus, Bacillus licheniformis, and Promicromonospora sp. (Richardson et al. 2009; Figueiredo et al. 2010; Vacheron et al. 2013). According to Ambawade and Pathade (2015) and Ahemad and Kibret (2014), Bacillus siamensis can generate GA and promote the development of banana plants. The primary GA type found in Azospirillum, GA3, seems to have a role in fostering plant development (Cassanet al. 2009).

1.1.4 Cytokinins: Cytokinin helps plants develop and differentiate their cells (Zhao et al., 2024). They benefit the plant by preventing tissues from aging, which affects leaf development.Cytokinins down-

regulate the auxin-induced apical dominance (Sipes and Einset, 1983). An developing alternative to boost plant development and increase agricultural yield and quality is the production of cytokinins in soil by PGPR (Mughal et al., 2024).

1.1.5 Ethylene and Abscisic acid: Roots excrete 1-aminocyclopropane-1-carboxylate (ACC), a direct precursor of ethylene. The PGPR-produced enzyme ACC deaminase transforms ACC into α-ketobutyrate and ammonium (Hayat et al. 2010; Glick 2014). Ethylene reduces the negative effects of biotic and abiotic stressors at low concentrations (Glick 2014). Abscisic acid (ABA), like ethylene, regulates plant growth in stressful environments (Glick 2015; Spence and Bais 2015). Bacillus licheniformis, Pseudomonas putida, and Achromobacterxylosoxidans are ABA-producing bacteria that have a positive impact on plant homeostasis (Sgroyet al. 2009; Glick 2014).

1.1.6 Phosphate solubilization: Phosphorus is the second most essential macronutrient needed by plants, after nitrogen (Sen et al., 2024; Saber et al., 2005). Although the typical soil contains around 0.05% (w/w) of P, only 0.1% of the total P is accessible due to fixation and poor soil solubility (Ducousso-Détrez et al., 2024). Microbial phosphate solubilization is known to involve a wide range of bacteria (including Pseudomonads and Bacillus), fungal strains (including Aspergilli, Penicillium, and Rhizoctoniasolani), yeasts (including Yarrowialipolytica and Pichia fermentans), actinomycetes (including Streptomyces and Actinomyces), cyanobacteria (including Anabeanasp, Calothrixbraunii, and Scytonema sp.), and arbuscular mycorrhizal fungi (including Glomus fasciculatum) (Saha et al., 2024; Bai et al., 2024; Sharma et al, 2013).

1.1.7 Siderophore production: Another characteristic of rhizobacteria is the generation of siderophores (Bisht et al., 2018). According to Ramos Cabrera et al. (2024), these compounds are effective in sequestering Fe3+ ions, which are thought to be crucial for metabolism and cell proliferation. According to Kumar et al. (2015), the microbes that live in plant roots compete with one another for the available iron in the soil and may prevent the growth of other harmful bacteria in the rhizosphere.

1.1.8 Biological nitrogen fixation: One of the main factors limiting plant development is the availability of nitrogen, which is needed in the greatest quantity (Courty et al. 2015; Bhattacharjee et al. 2008; Figueiredo et al., 2013). Anabaena sp., a free-living diazotrophic nitrogen fixer bacterium, and Azolla work together to make up for the nitrogen deficiency in a waterlogged rice field; for this reason, Anabaena is sometimes called a "biofertilizer" (Bhuvaneshwari and Kumar 2013; Fosu-Mensah et al. 2015). Thus, Anabaena increases plant growth, decreases nitrogen loss by ammonia volatilization, and supplies large amounts of nitrogen (up to 50 kg ha−1) (Bhuvaneshwari and Kumar, 2013).

1.2. Indirect mechanisms

1.2.1Biocontrols: Broad-spectrum antibiotics, lactic acid from Lactobacillus lactis, hydrolytic enzymes like lysozymes, chitinase, proteases, lipases, and β,1-3, glucanases that lyse the pathogenic microorganisms, and various exotoxins and bacteriocins that also have bactericidal activity are just a few of the many antimicrobial compounds that PGPR can produce for defense (Riley and Wetrz, 2002).

1.2.2 Antimicrobial compounds: Antibiotics are a diverse class of low molecular weight, organic substances that negatively impact other species' development or metabolic processes. (Kwon and Kim 2014; Sharma et al., 2024; Duffy, 2003). However, bacteriocins have a somewhat limited bactericidal range, meaning they are primarily harmful to strains that are closely related to the ones that produce them (Riley and Wetrz, 2002).Although bacteriocins are produced by strains of Leuconostocmesenteroides subsp. dextranicum, the chemicals involved have not yet been fully isolated and described (Angelini, 2024; Chen et al., 2024).

1.2.3 Stress tolerance: The metabolism involved in the rhizosphere environment's production of phytohormones (Araujo et al. 2005) and the induction of resistance to biotic and abiotic stresses (Kang et al. 2010) are two prominent metabolic pathways that have been studied and in which rhizobacteria participate. Proline buildup in plants functions as an osmoprotectant, preserving the water potential in situations of shortfall and promoting soil water absorption (Hanson et al. 1979).

1.2.4 Antioxidant enzymes

A variety of antioxidant enzymes that scavenge free radicals, including catalases, peroxidases, and superoxide dismutases, are produced by plants (Simova-Stoilova et al., 2008). Plants can produce a significant amount of antioxidant enzymes when growth-promoting bacteria are introduced to the rhizosphere (Araujo and Menezes, 2009; Kumar et al. 2009). According to Moraes and Colla (2006), antioxidants can either directly or indirectly counteract the effects of free radicals by participating in the enzymatic systems that carry out this activity. Flavonoids, which function as strong antioxidants and metal chelators, are the primary antioxidant chemicals with nutraceutical properties found in plants

(Tapas et al., 2008).

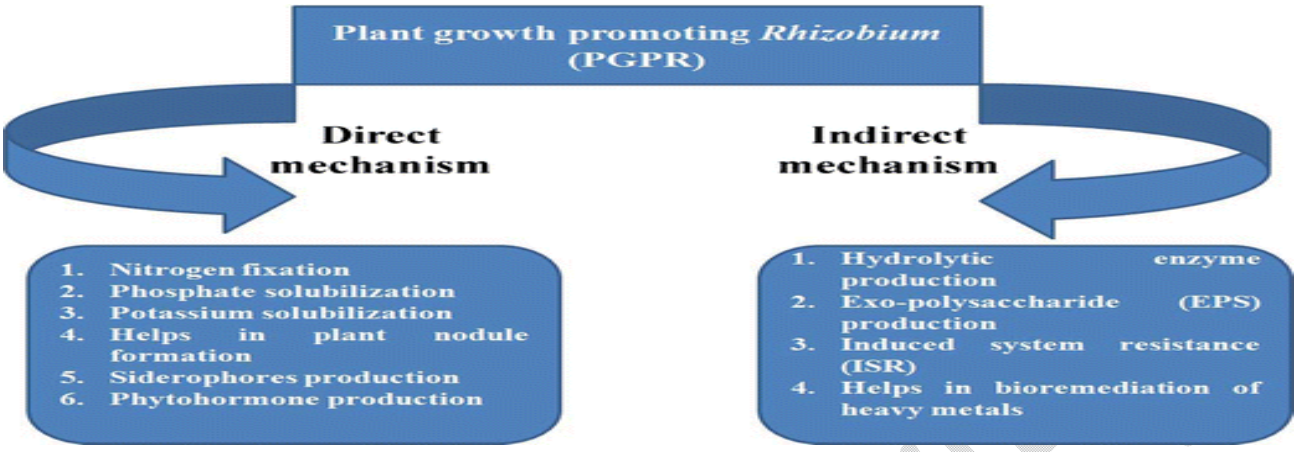

Fig:1 Mechanisms of plant growth promotion by PGPR(Du, et al., 2020).

2. Identification and characterization of beneficial soil microbiota

Colwell (1970) used the phrase "polyphasic taxonomy" to describe molecular, biochemical, and phenotypic detection techniques. Understanding the phenotypic characteristics of an unknown microbe, such as its morphological, cultural, and biochemical traits, is the first step in identifying it (Bisht et al., 2019).

2.1. Phenotypic characterization

Different types of microorganisms have different cell sizes, shapes, and arrangements (Compant et al., 2024). Table 2 lists the sizes of a few microorganisms. There are three fundamental morphological types of bacteria. (Shreshtha et al., 2024) Coccus, Bacillus, and Spiral (Fig. 2). Hyphae are thin-walled, heavily branching filaments that are formed by some multicellular bacteria (Arellano-Wattenbarger et al., 2024; Banick et al., 2024; Fonseca-García et al., 2024).Cultural traits, 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), can also be used to identify microorganisms.(Woźniaket al., 2019; Sangeetha &Thangadurai, 2013)

Table 2 Phenotypic and physiological features of some common bacteria(Woźniak, et al., 2019)

Organisms	Significance	Size (µm)	Morphology

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 *Clostridium*	80 x 600	Rods with tapered end

Lyngbya majuscule	Produce altillatoxin and kaklitoxin, causal organism of seaweed dermatitis	8 x 80	Filaments
Magnetobacteriumbavaricum	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
Thiomargaritanamibiensis	Largest bacteria ever discovered	750	Cocci in chains
Thiovulummajus	Sulfur oxidizing, fastest swimming bacteria with the speed of 615 µm/sec	18	Cocci

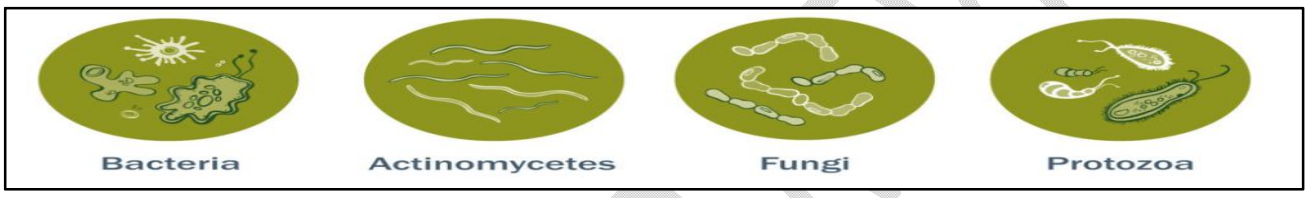

Figure 2 Morphological features of different microorganisms (AgSolutions; Australia; https://agsolutions.com.au/soil-microbes-sustainability/)

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

Pulse Field Gel Electrophoresis (PFGE), Amplified Ribosomal DNA Restriction Analysis (ARDRA), and Restriction Fragment Length Polymorphism (RFLP) are among the methods (Fusco et al., 2023; William et al., 1990).According to Dabban et al. (2024), RFLP is a preliminary genetic study that enables individuals to be identified based on their distinct pattern of restriction enzyme cutting in certain

DNA regions. According to Monteiro et al. (2024), AFLP employs particular adaptors that are ligated to enzyme-restricted DNA. Primers from the adaptor and restriction site-specific sequences are then used to amp up the DNA.ARDRA uses several restriction enzymes to digest amplified ribosomal DNA, and a profile is produced by combining these patterns (Kalia and Parshad, 2013; Maslow, 1993).In order to liberate DNA and shield the chromosomal DNA from mechanical damage, PFGE employs in situ lysis of bacterial entire cells in agarose plugs (Bashbosh et al., 2024; Dubey et al., 2024; Qurbani et al., 2024).Because of its widespread distribution and high degree of conservation, the 16S rRNA gene sequence is therefore utilized to infer the phylogenetic relationships among bacteria (Hammoudeh et al., 2024; Botan et al., 2024). The 16S rRNA gene sequence is then aligned using sequence-comparing software programs like BLAST and CLUSTAL X. (Bisht and others, 2022; Li and others, 2024).Fig. 3 summarizes the characterization of recently identified strains using several molecular techniques.The foundation of DNA-DNA hybridization is a comparison of two bacterial species' whole genomes (Ansari et al., 2024; Pasquer et al., 2010).

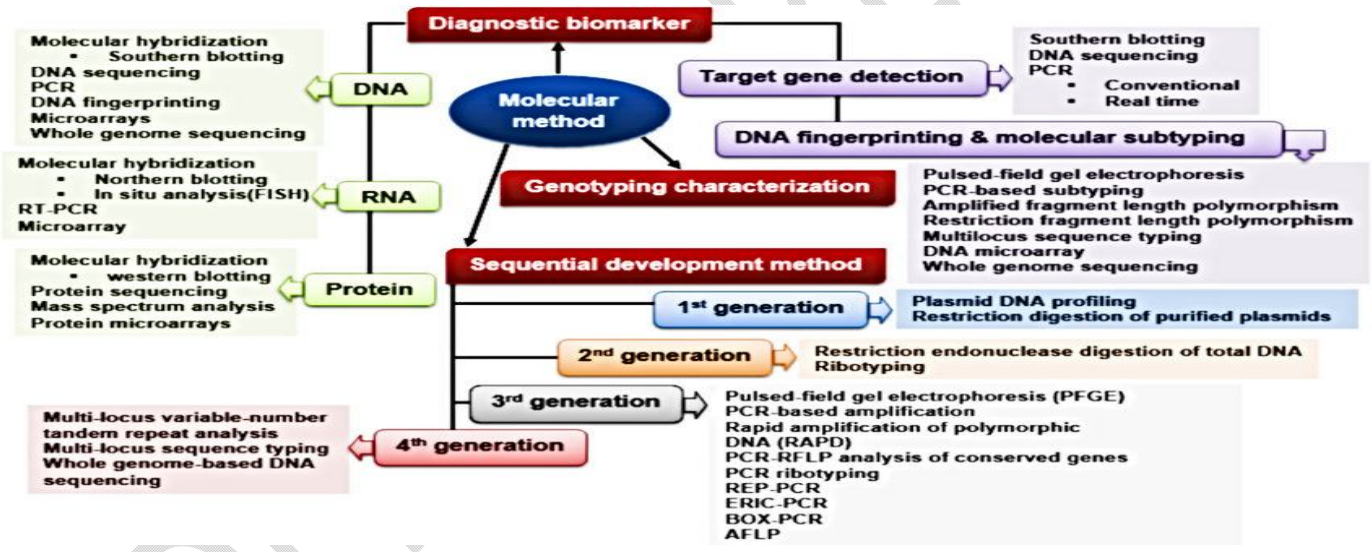

Fig. 3 Characterization of newly isolated strains by different molecular methods(Haque et al., 2021)

2.3.2Other advanced molecular techniques

a. FISH (Fluorescence In Situ Hybridization)

b. In situ fluorescence (FISH) and chromogenic (CISH) detection techniques are employed to visualize RNA and DNA targets (Román-Lladó et al., 2024).Non-isotopic dyes took the role of radioactive labels when fluorescent labels were developed (Landegent et al., 1984; Pinkel et al., 1986; Pinkel et al., 1988). To identify individual microbial cells, DeLong initially employed fluorescently tagged oligonucleotides in 1989 (Aggarwal et al., 2024; Kliot and Ghanim, 2016; Luongo et al., 2024; Tseng et al., 2024). Horseradish peroxidase (HRP), which uses fluorescein-tyramide as a substrate,

was utilized to label oligo nucleotides (Herr et al., 2015; Ye et al., 2014; Yan et al., 2022). The use of polyribonucleotide probes tagged with several fluoro chrome molecules greatly enhanced the sensitivity of FISH in natural samples.

c. Microautoradiography

A radiotracer is used for incubation of sample to label microbial cell using radioisotopic compounds like soft beta emitters (^{3}H, ^{14}C, ^{33}P) or strong beta emitter like ^{32}P**(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. 4**shows stepwise procedure for microautoradiography**(Nielsen and Nielsen, 2005)**.

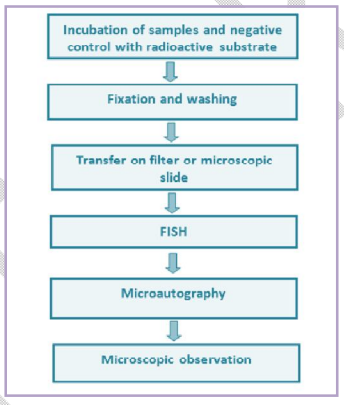

Figure 4: Step wise procedure of microautoradiography

d. Sequencing technology

Longer sequences are broken up into smaller pieces that can be sequenced independently due to the size limit of Sanger sequencing, which is 100–1000 base pairs (Muñoz-Ramírez et al., 2024; Siew et al., 2024; Li et al., 2024).Because of its low error rate, high read length (> 700 bp), and bigger insert sizes (i.e. > 30 Kb), Sanger sequencing is still utilized for sequencing (Chen et al, 2014; Sanger et al., 1977; Soodet al., 2008; Zaytsevetal., 2024).The Illumina/Solexa and 454/Roche systems are two NGS technologies that may be used on metagenomic material (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/Solexatechnology immobilizes random DNA fragments on a surface and then performs solid surface PCR amplification, resulting in clusters of identical DNA

fragments**(Chettri, et al., 2024; Minoche*et 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 procedureinclude—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)**.

e. **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)**.

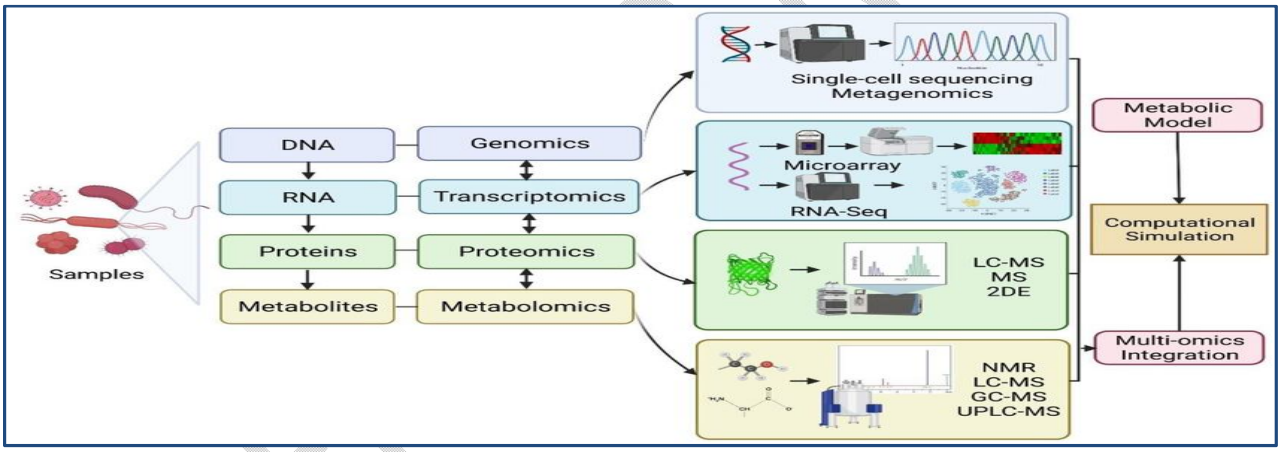

Figure5:Schematics representation of omics dogma describing the network the processing of DNA, RNA, proteins and metabolites and method used(Yow et al., 2022).

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

According to Ejaz et al. (2024) and Yu et al. (2024), metagenomics is a culture-independent method for analyzing the whole genome of microbial communities found in a specific environmental sample.According to Debnath et al. (2010) and Devi et al. (2024), phenols and humic acid obstruct downstream processes like PCR amplification and gene cloning (metagenomic library creation).Pace was the first to suggest the direct cloning of DNA from environmental materials (Pace et al., 1985; Schmidt et al., 1991; Healy et al., 1995). Libraries made from prokaryotes found in saltwater were reported by DeLong's group (Stein et al., 1996; Suwannaphan et al., 2024).

1.2 Transcriptomics:

According to Aslam et al. (2024), transcriptomics is the comprehensive scanning of the approximately 50,000 known genes that are translated into mRNA from the three billion-letter human genome. This novel method has the benefit of analyzing the entire transcriptome—the entire collection of all mRNA molecules present at a certain moment in a given cell population—in a single analysis rather than one gene at a time (John Martin et al., 2024; Vohra et al., 2024). The majority of proteins and all genes may be viewed as tools for creating an organism's biochemical makeup and, consequently, its physiological identity (Raza et al., 2024).

1.3 Proteomics

Studies at the protein level are essential to shed light on the molecular mechanisms driving growth, development, and their interactions with the environment since proteins are the most significant constituents of major biochemical and signaling pathways (Pan et al., 2024; Macaulay et al., 2005). All of an organism's genes, which encode the proteins needed for its function, are found in its genome. The thorough study of proteins and their biological roles and activities is known as proteomics (Kaya, 2024; Siddiqui and Saeed, 2024).To identify genes that are comparable to those previously known, the genome sequence of one creature can be compared to that of other organisms (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)**.

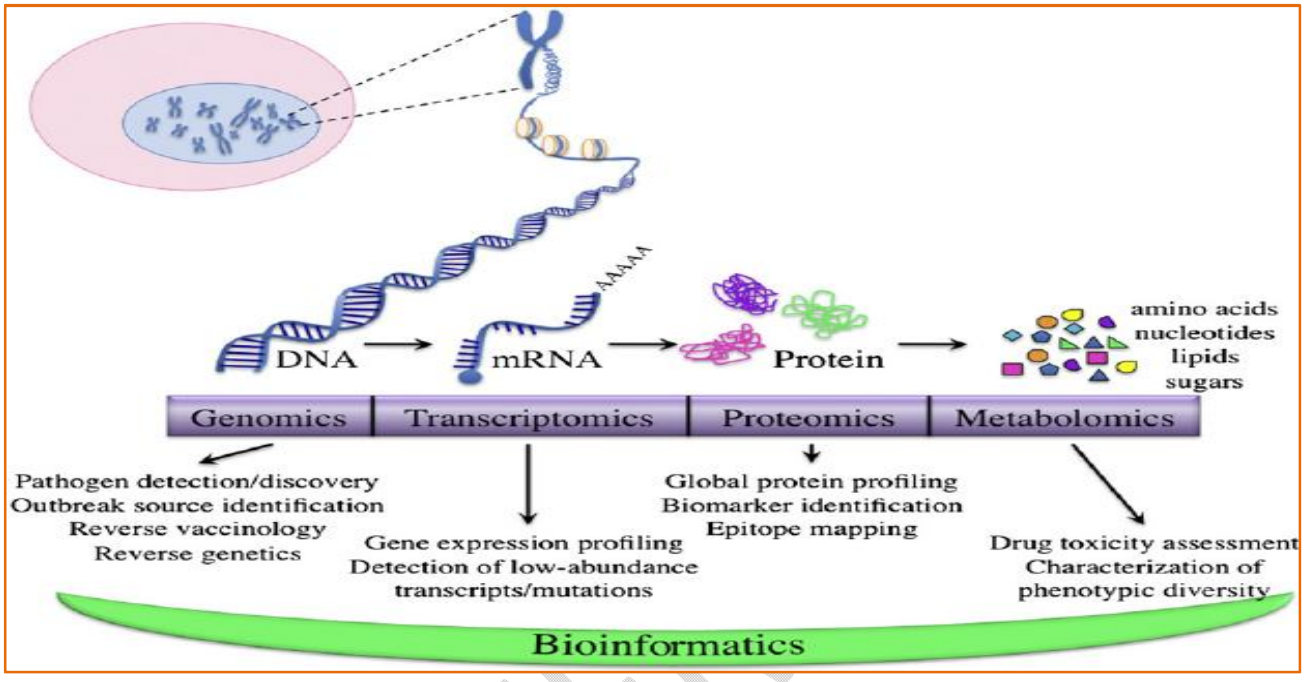

Figure 6:Integrating omics technology:genomics, proteomic, transcriptomic, and metabolomic(Fontana et al., 2012)

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)**.

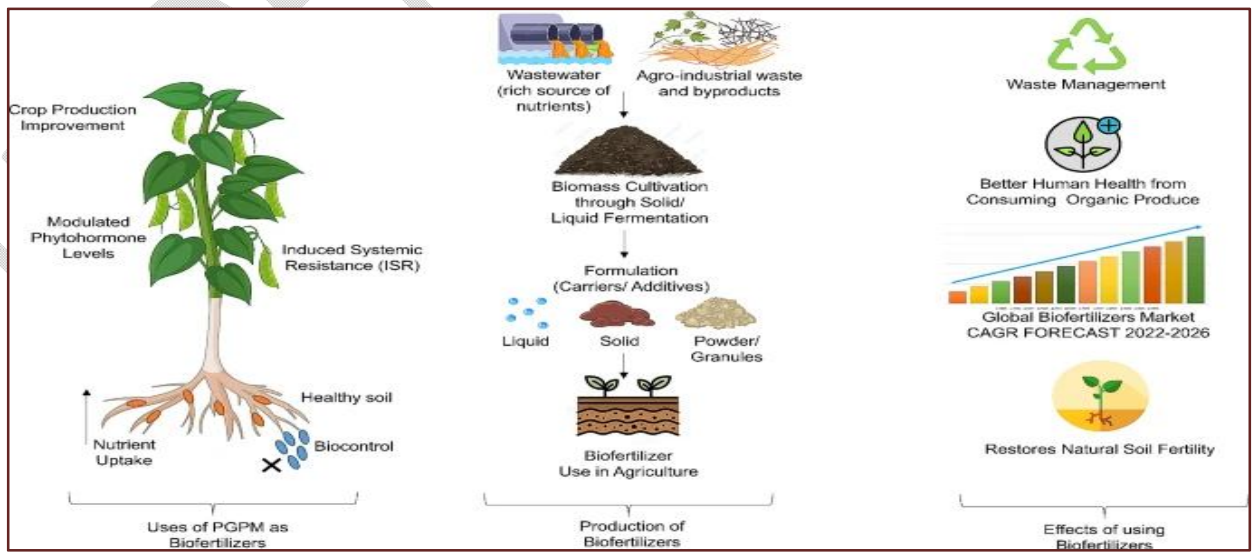

Figure7:Commercial biofertilizers manufacturing process(Bagga et al., 2024)

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

Divergent opinions exist about biofertilizers. Because of the lack of technology and expertise to create biofertilizer products from plentiful waste, it is frequently thought to be more costly than chemical fertilizers. In addition, compared to chemical fertilizers, the impact on the crops is gradual. To keep the microbial inoculum functional for a long time, it must also be handled with special care, such as storage or combining with powders. Because biofertilizers include live microorganisms, environmental factors affect how successful they are. As a result, uneven results are inevitable. To achieve successful inoculation, biofertilizer bottlenecks still need to be addressed, including short shelf life, lack of appropriate carrier materials, vulnerability to high temperatures, and issues with storage and transportation.

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,integratedomics, 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.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies use during written this paper such as Large Language Models (ChatGPT, COPILOT, etc.)

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