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Bioinoculants: the agrarian avengers

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Abstract

Bioinoculants are beneficial microorganisms that are used in agriculture to enhance plant growth and productivity, improve soil health, and reduce the use of chemical fertilizers and pesticides. They include bacteria, fungi, protozoa, and endophytes that interact with plants in various ways to promote growth, nutrient uptake, and stress tolerance. The interactions between bioinoculants and their host plants are complex, and different strains of bacteria, fungi, and protozoa have specific interactions with different plants. Understanding these interactions is critical in selecting the appropriate bioinoculant for a particular crop and soil type. This paper reviews the interaction of different types of bioinoculants with plants, and their potential to improve the sustainability of agriculture and their applications. Techniques for applying bioinoculants include seed treatment, soil application, and foliar application. Bioinoculant application has been shown to improve crop yield, quality, and nutrient content. In addition, they help to reduce environmental pollution and protect soil biodiversity. Some of the challenges associated with the application of bioinoculants include the need for optimized formulations, storage, and transportation. To maximize the potential of bioinoculants in sustainable agriculture, it is necessary to continue research into their interactions and develop effective application techniques that can be used on a large scale.

Keywords Bioinoculants · Symbiosis · Soil-microbe interactions · Soil biodiversity · Sustainable agriculture

1 Introduction

Over two decades the practice of good sustainable agriculture has been a priority in research. The extensive use of chemicals for higher crop yields has led to a gradual depression in the soil and agricultural quality. The continuously growing population has a major demand of satisfying hunger. For this, it is essential to develop and practice new eco-friendly, and long-lasting higher crop-yielding techniques. In agriculture, proper nutrient availability is important to get sufficient yield. Microorganisms play a very crucial role in nutrient cycling being a widely held part of any ecosystem. Also, it is an eco-friendly approach to enhancing crop productivity without compromising the quality of the soil. The interaction between the microorganisms and other abiotic and biotic factors is a fundamental stage for comprehending the function and relationship of microbial networks (Imam et al. 2016).

Although, the evolution in farming started with commodities inputs and working up the supply chain to food retailing and distribution. Famine spread over South Africa in the summer of 2002, Angola, Malawi, Zambia, and Zimbabwe, as well as a substantial number of people in Lesotho, Mozambique, and Swaziland were in grave danger of going hungry. According to "*Save the Children*", roughly 9.4 million people are in urgent need of food, with fears that this figure could climb to almost 16 million by the beginning of 2003 (Coleman et al. 2023). Also for the production and restoration of food, the global bodies set the rule and conditions namely, the Food and agriculture organization (FAO), and Biodiversity International (IPGRI) as this review are discussing the role of bioinculants involving microorganisms.

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2 Bioinoculants and their interaction

2.1 Bacteria

Soil sustains a large variety of terrestrial microorganisms including bacteria, archaea, fungi, and other microorganisms. These microorganisms colonize the rhizosphere as well as the plant tissues and help with promoting plant growth by providing them with essential minerals and nutrients from the soil. Bacteria constitute a large fraction of the soil microbiota, especially in the rhizosphere. Soil bacteria play an important role in crop processing because they participate in processes such as providing soil nutrients, increasing plant growth, regulating or inhibiting plant diseases, enhancing soil composition, bioaccumulation, and inorganic microbial leaching (Shah et al. 2021). Biocontrol, plant growth promotion, stem degradation, phosphorous solubilization, humification, and nitrogen fixation along with phytohormone production, and induction of defense mechanisms are all microbial abilities that can be used to build microbial soil inoculants for use in sustainable agricultural production (Allaga et al. 2020; Suman et al. 2016), where they are directly added to the soil or used to coat seeds before sowing (Owen et al. 2015). Several different kinds of microorganisms such as Cyanobacteria (*Anabaena, Nostoc, Aulosira),* are capable of fixing nitrogen, whereas some bacterial genera such as *Pseudomonas, Bacillus, Burkholderia, Acidothiobacillus* and *Paenibacillus* are capable of converting insoluble forms of minerals such as potassium into soluble forms making their uptake easier for the plants (Malusà et al. 2016).

Plant growth-promoting rhizobacteria (PGPR), being excellent biocontrol agents as well as having plant growth-promoting abilities, are a potential candidate which can be used as an alternative to the toxic and polluting agrochemicals (Orozco-Mosqueda et al. 2021). PGPR employs various mechanisms including the acquisition of nutrients and minerals, enzymes and antibiotic production, and stimulation of phytohormone production to promote the growth of the plants (Orozco-Mosqueda et al. 2021). Nitrogen is a macronutrient essential for the growth and development of plants along with seed and fruit production, which is used by plants to synthesize amino acids, vitamins, nucleic acids, and other nitrogenous compounds (Fasusi et al. 2021; Orozco-Mosqueda et al. 2021). Free-living, as well as symbiotic nitrogen-fixing bacteria, have been found to promote plant growth by increasing nitrogen uptake in plants via biological nitrogen fixation (Malusà et al. 2016). Symbiotic *Rhizobium sp.* and genera including *Bradyrhizobium, Sinorhizobium, Mesorhizobium, Rhizobium, Azorhizobium, Neorhizobium,* and *Pararhizobium* are rhizobacteria that penetrate the root of leguminous plants and other plant tissue following chemical signals secreted by the plants in the form of flavonoids, and form nodules where these microbes reside and convert the atmospheric nitrogen into ammonia, a form assimilable by the plants via biological nitrogen fixation (Fasusi et al. 2021). Free-living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum* and certain non-leguminous nitrogen-fixing bacteria such as *Achromobacter, Acetobacter, Clostridium, Azomonas, Bacillus, Erwinia, Enterobacter, Desulfovibrio, Corynebacterium, Campylobacter, Klebsiella, Mycobacterium, Rhodospirillum, Rhodo – pseudomonas* also fix the substantial amount of nitrogen for the plants to utilize (Nosheen et al. 2021; Shah et al. 2021). These microbes can be incorporated into the soil or with seeds as bioinoculants to enhance the nitrogen uptake ability of the plants. Bacteria produce many metabolites that help in plant defense and growth. There are various phenomena like Quorum Sensing, computational biology techniques, Crispr Cas9 that can be used to improve and engineer the efficiency of bioinoculants to accelerate plant defense and growth (Kamath et al. 2022, 2023; Parmar et al. 2020; Shukla et al. 2021).

Phosphate is another nutrient important for plant growth and development, which is usually present in insoluble form in the soil, thus, limiting its availability to plants. However, certain soil microbes can convert the insoluble form of phosphate into a soluble form. Phosphate is found as insoluble compounds (apatite) or in organic forms (inositol phosphate, phosphomonoesters, and phosphodiester) in the soil and is unavailable to plants (Orozco-Mosqueda et al. 2021). Moreover, it leaches into the ground polluting the groundwater reservoir (Orozco-Mosqueda et al. 2021). Phosphate-solubilizing bacteria (PBS) use different mechanisms such as organic acid production, production of H_2S, ion exchange reaction, and chelation for the solubilization of phosphates (Mahanty et al. 2017; Nosheen et al. 2021; Sharma et al. 2013). Production of these organic and inorganic acids promotes the solubilization of other minerals such as zinc and potassium which are essential for crop improvement (Orozco-Mosqueda et al. 2021). A multitude of phosphate-solubilizing bacteria are present in the soil to carry out these functions, their population composition depends on various properties of the soil. *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Aspergillus, Penicillium, Fusarium, Sclerotium, Enterobacter, Rhizobium,* etc. are some of the most common PSB found in the soil (Anand et al. 2016). Amongst them certain species of *Bacillus* such as *Bacillus megaterium, B. subtilis, B. polymyxa, B. circulans, B. sircalmous,* and *Pseudomonas* and *Enterobacter* are considered to be the most important bacterial strains involved in phosphate solubilization (Anand et al. 2016). Exopolysaccharides produced by microbes also help in plant growth and protect them from abiotic stresses. Exopolysaccharide producing bacteria can be used as bioinoculants aiming at enhanced plant growth and resistance against abiotic stresses (Shukla et al. 2019).

Iron is one of the most abundant elements found in the earth's crust, however, it is in the least assimilable forms. Microorganisms found in association with plants produce a siderophore for the acquisition of iron. Siderophore is an iron chelating compound that reduces iron intra- and intercellularly for the use of both plants and the microbe (Orozco-Mosqueda et al. 2021). Other than providing the plants with iron, microbial siderophore also defend the plants against pathogens by competing with them for iron. Microbial species such as *Pseudomonas fluorescens* produce Fe-siderophore complexes that can be taken up by plants to acquire iron as per their needs (Parray et al. 2016).

Microbes capable of producing phytohormones and volatile organic compounds known to stimulate plant growth are potential candidates as bioinoculants. Phytohormones like auxins, gibberellins, cytokinin, IAA, and other volatile compounds participate in various plant growth processes such as seed germination, root formation, photosynthesis, cell elongation, etc. (Orozco-Mosqueda et al. 2021). PGPR along with some pathogens, symbiotic microbes as well as free-living rhizobacterial species have been found to produce phytohormones such as IAA and Gibberellic acid in the rhizosphere (Parray et al. 2016).

According to a study (Boiero et al. 2007), it has been observed that the gibberellin-like substance secreted by *Azospirillum* spp. Promoted growth in rice and maize dwarf mutants. Several microbial species such as *Aeromonas, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Pseudomonas, Rhizobium, Klebsiella, Enterobacter, Azospirillum* produce Indole Acetic acid (IAA) (Parray et al. 2016; Santoyo et al. 2019).

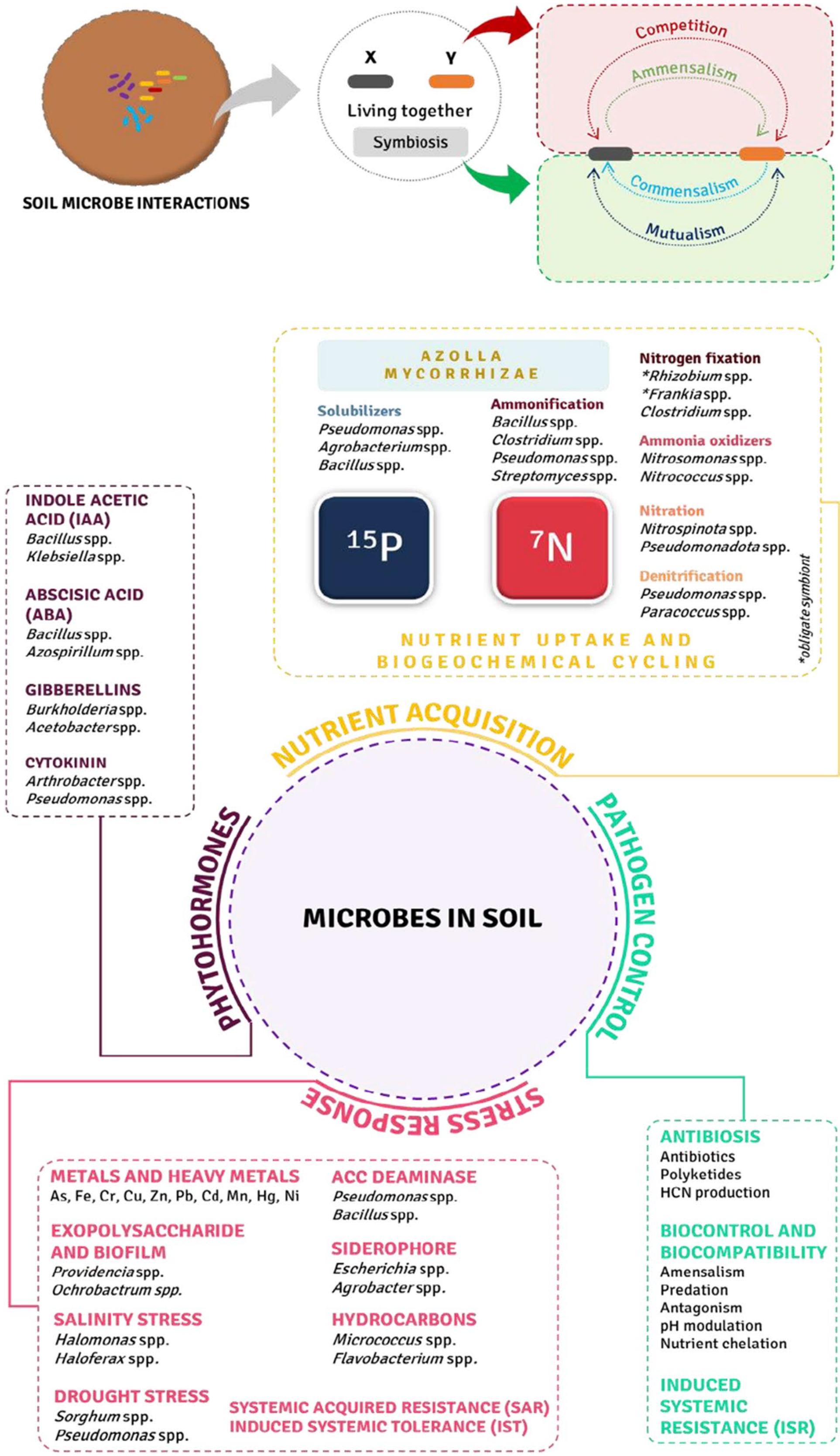
Microorganisms also use certain indirect mechanisms towards potential pathogens of plants by restricting the growth or eliminating the pathogens. Plant-associated microbes produce compounds and enzymes that help with plant defense against pathogenic microorganisms, such microbes have the potential to find use as bioinoculants. Siderophore production by rhizobacteria limits the iron resource availability for the pathogens along with providing iron to plants. Bacterial species such as *Bacillus, Pseudomonas, Streptomyces, Stenotrophomonas* and other PGPR bacteria have been found to produce antibiotics such as zwittermicin-A, pyrrolnitrin, phenazine-1- carboxamide, pyoluteorin, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, and cepafungins, oligomycin A, kanosamine, and xanthobaccin, etc. (Parray et al. 2016; Santoyo et al. 2019). Production of enzymes such as chitinase, cellulase, and glucanase by *Pseudomonas, Bacillus,* and *Sinorhizobium* suppresses the growth of pathogenic fungi (Fasusi et al. 2021; Santoyo et al. 2019). In a study, *Psuedomonas fluorescens* and *Sinorhizobium* have been found to control the Fusarium wilt of pigeon pea and soft rot in potato plant caused by the action of pathogens *Fusarium udum* and *Erwinia carotovora* (Raimi et al. 2017). *Pseudomonas aeruginosa* is being used to combat the rice crop disease caused by *Xanthomonas oryzae* and *Rhizoctonia solani* in West Africa (Alaux et al. 2018). Induced Systemic Response (IRS), a plant defense mechanism against phytopathogens induced by bacteria, can be induced in plants by the production of certain volatile compounds (such as acetoin) by bacterial species such as *Bacillus* spp. (Santoyo et al. 2012) (Fig. 1).

2.2 Fungi

Fungi are eukaryotic, non-motile, and non-vascular organisms. As their heterotrophic characteristics, Fungi have both positive as well as a negative impact, plants. It has a great significant role in agriculture as well as in agroforestry (Rangel et al. 2018). As their positive impact on fungi, it has an essential role, to prevent and control of plant disease by potentially use of biocontrol agents. Through the mycelium, fungi absorb nutrients from the surrounding environment in two different stages. A Breaking down of biological polymers into small units like monomers. These monomers are absorbed into the mycelium by the process of facilitated diffusion and active transport processes. Through this process, fungi help to promote plant and helps to increase plant defense mechanisms, plant disease, and terrestrial weeds (Rangel et al. 2018).

Current studies agriculturally focus on Mycorrhizal fungi. Fungi play a significant role in the organic matter recycling process (Pathak and Kumar 2016). They establish via symbiosis relationship with the roots of plants. Therefore, they are divided into 2 major types. Endomycorrhizal fungi and Ectomycorrhizal fungi (ECM). Endomycorrhizal fungi form symbiosis relationship that they impale into the plant cell, to exchange nutrients through the roots of plants as well as they also form intracellular colonization and produces branch hyphae, which is also called arbuscles, inter part of the cells in the plant root of the cortex. example Arbuscular fungi (VAM/AM). whereas Ectomycorrhizal fungi by symbiosis relationship with plants form hyphae sheath surrounding the plant root surface and cultivate between the plant cells and swap the nutrient with each other. As AM fungi help to regulate the growth of the plant as well as reduce the chance of the harmful effects of plants exposed to salt stress. With AM fungi, there are number of microorganisms that improve plant growth and yield under such various stress conditions. To cope with that AM fungi, play an essential key role in reducing the toxicity induced by salt stress, consequently, they uptakes a mechanism in plants by supplying the essential nutrient in this wise, plants can recover the water balance machinery, enhancing their tolerance capacity,

Fig. 1 Soil microbe interactions. The diagram represents the diverse components and essential role of soil microbes



and thereby enduring the salt stress (Hameed et al. 2014; Carretero et al. 2008). Trichoderma, which is an example of non-mycorrhizal fungi, is well-known for influencing plant stress reactions and has the ability to capable of colonizing or antagonize harmful fungi by activating plant defensive responses and enhancing plant development.

Most terrestrial plants benefit from mycorrhizal fungi connections because they provide improved nutrient access and resistance to abiotic and biotic stress (Tedersoo et al. 2020). Mycorrhizal fungi, which include pathogens and mycorrhizosphere mutualists that fix atmospheric nitrogen, take up phosphorus, produce vitamins, and/or protect against antagonists, mediate plant interactions with the soil microbiome, including pathogen and mycorrhizosphere mutualists that fix atmospheric nitrogen, take up phosphorus, produce vitamins, and/or protect against antagonists. Bioinoculants also help the plants withstand adverse conditions. In the study reported by Patel et al. (2012), plant growth promoting rhizobacteria protected the chickpea plants from salinity stress which resulted in amplified growth and resistance to salt stress. Mycorrhizal root symbionts, together with other biotic and abiotic factors, control plants allowing access features, influence plant-plant interactions, and modify ecosystem processes through these roles. In soil, extensive shared mycorrhizal networks connect conspecific and heterospecific plant individuals, regulating nutrient transfer and sending phytochemical signals (Tedersoo et al. 2020). Interlinking hyphal networks, they (Tedersoo et al. 2020) believe, have a synergistic effect on plant communities and ecosystem services by altering the functional characteristics and autecology of host plants. This is certainly most evident in orchids and myco-heterotrophs' specialized C feeding. Plant population and community ecology are influenced by different forms of mycorrhiza, which affect plant dispersal, establishment, and coexistence. The distribution of OM (orchid mycorrhiza) and EcM (ectomycorrhiza) plants is restricted more than that of AM, ErM (ericoid mycorrhiza), and NM plants attributed to dispersal limitations of one or both symbiotic partners. Eventually, species-specific potential benefits and resource exchanges may be key mechanisms by which a diverse range is passed on in mutualistic plant-mycorrhizal fungus systems.

2.3 Actinomycetes

Actinomycetes are gram-positive, aerobic, and saprophytic bacteria belonging to the order Actinomycetes actinomycetes known for their mycelium production. This group of bacteria interacts with non-leguminous plants and participates in the process of nitrogen fixation. Actinomycetes are ubiquitous and can be found in soil, water, and atmosphere as well as endophytes (Solanki et al. 2016). The members of the actinomycetes group especially those under *Streptomyces* have been found to produce antibiotics and antifungal compounds as secondary metabolites (Běhal 2000). This group of microorganisms produces a variety of bioactive compounds and enzymes that could potentially improve crop growth, health, and yield when employed as bioinoculants. Actinomycetes are capable of carrying out processes like phosphate solubilization, production of phytohormones, enzymes, and antibiotics.

The process of phosphate solubilization is carried out by actinomycetes acidification of the media or by the production of a chelating compound that speeds up the process of phosphate solubilization (Lacava and Sousa 2016). Several strains of *Actinomycetes* were isolated and studied for phosphate solubilization, out of which *Streptomyces cavourensis, S. griseus, Micromonospora aurantiaca* were found to have high P-solubilizing activity (Gangwar et al. 2012). Actinomycetes have been known to promote plant growth and development through the production of phytohormones such as IAA or siderophores to quench iron from surrounding soil to help with nutrient uptake (Lacava and Sousa 2016). Some of the IAA-producing actinomycetes include *Actinoplanes campanulatus, Micromonospora chalcea*, and *Streptomyces spiralis* which has shown growth enhancement in cucumber plants (El-Tarabily et al. 2009).

Actinomycetes produce certain enzymes such as chitinase, pectinase, xylanase, cellulase, amylase, protease, lipase, and β-1,3-glucanase. The lytic enzymes chitinases and glucanase degrade the chitin and glucans constituting the cell wall of fungal pathogens such as *Fusarium oxysporum, Sclerotinia minor, S. rolfsii*, and *Aspergillus*, *Phytophthora* (Solanki et al. 2016). A multitude of antibiotics and antifungal compounds such as actinomycin X2, fungichromin produced by members of *Actinomycetes* facilitates the inhibition or elimination of bacterial as well as fungal phytopathogens (Solanki et al. 2016). According to a study carried out by Hwang et al. (Hwang et al. 2001) phenylacetic acid and sodium phenylacetate produced by *Streptomyces humidus* shows antimicrobial activity against *Rhizoctonia solani, Pythium ultimum, Phytophthora capsica, Saccharomyces cerevisiae,* and *Pseudomonas syringae* pv. *Syringae*. It has also been reported that actinomycetes produce antifungal antibiotics such as polyoxins A and B from *Streptomyces cacaoi* var. *asoensis*., mildiomycin from *Streptoverticillium rimofaciens* B-98891 (Iwasa et al. 1978), tubercidin from *Streptomyces viola-ceoniger* (Hwang and Kim 1995), oligomycin A and C isolated from *Streptomyces diastaticus* (Yang et al. 2010), against several fungal plant pathogen. Other than antifungal and antibacterial compounds, actinomycetes have been reported to produce compounds with insecticidal activity. Therefore, actinomycetes can be used as microbial pesticides to protect plants and crops against insect pests (Solanki et al. 2016).

2.4 Protozoa

Protozoa are unicellular eukaryotic microbes that lack a cell wall and exist in the Protista kingdom. A trophozoite is a vegetative, reproducing, feeding form of a protozoan, and some protozoa generate a protective form termed a cyst under particular conditions. From earthworms and tiny soil animals to fungi and bacteria, the soil food web is made up of species of various sizes and activities. As a primary source of energy, soil organic matter, whether derived directly from plants or animals, drives the soil food web.The soil microflora (bacteria, fungus, and protozoa) degrades organic matter, whereas the soil fauna, and their interactions with other soil organisms, have an impact on nutrient (N, P, and S) cycles.While microfauna consumes microflora directly, mesofauna consumes detritus, which is rich in microflora and is thus important in microbial turnover (both beneficial and harmful) and nutrient cycling.Macrofauna is renowned as ecosystem engineers because they break plant leftovers, causing microbial activity to increase. They can help redistribute organic matter and microbes by creating bio pores, especially when there is less agriculture and crop residue retention.

2.5 Microfauna- nematode communities

Worms can be found in all ecological niches, and there are rough as many nematode species (Phylum Nematoda) as insect species in nature. Phytopathogenic nematodes promote plant growth and use lytic enzymes and the thrusting of their disease caused by infection (needle-like mouthparts) to breach cell walls. Ectoparasitic nematodes and endoparasites nematodes are among them. The majority of soil nematode species are not plant-parasitic, but feed on other soil organisms and are thus classified as free-living. They are classified into distinct trophic groups based on their ability to feed on diverse microorganisms, such as bacterivores, fungivores, predatory nematodes, and omnivores, which can be predatory but can also feed on bacteria and fungi in the lack of suitable prey. Feeding on microflora and protozoans by free-living nematodes leads to the release of extra inorganic nutrients, notably N, that may be used by crops, hence accelerating nutrient cycling in soils.

In this research article (Gupta et al. n.d.), the researcher demonstrates that severely, inorganic fertilizers, soil moisture, organic matter additions, and manipulation were the most relevant elements impacting nematode populations in a comprehensive investigation. Whereas, Bacterivores are tiny free-living nematodes with short generation periods that respond fast to changes in soil bacterial populations caused by organic substrate inputs or soil disturbance. In the case of Fungivores have relatively long generation periods and are more receptive to saprophytic fungal populations that form on plant debris and mycorrhizal hyphae. Whereas Predatory and omnivorous nematodes have the biggest bodies and the longest generation periods, and they respond to increases in microbe-feeding nematodes and protozoans, they are also the most vulnerable to agronomic soil ecosystem changes.

3 Suppression of plant-parasite nematodes

Organic matter inputs stimulate a variety of soil microbiota that prey (e.g., predatory nematodes) and parasitize (e.g., bacteria and fungi) on nematodes, resulting in a general suppression of plant parasitic nematodes in a soil (Gupta et al. n.d.). Other specialized nematodes, such as *Mononchus spp*. and are nematode predators. springtails and mites are examples of microarthropods. Nematophagous fungi destroy nematodes by colonizing them. The majority of these microorganisms are non-specific in terms of the nematode species they prey on or parasitize, preying on both free-living and plant parasitic nematodes. Pasteuria spp. are mycelial and endospore-forming bacteria that infect root lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Pratylenchus penetrans*).

Microarthropods are important intermediary members of the soil food web with a key role in the decomposition of crop residues and SOM, and accelerating the mineralization of plant nutrients (e.g., N and P) through the consumption of microbes. Microarthropods are crucial intermediary components of the soil food web, playing a vital part in the decomposition of crop wastes and SOM, as well as speeding up the mineralization of plant nutrients (e.g., N and P) through microbe ingestion. They can also devour pathogenic fungi and AMF spores and hyphae, as well as aid in the dissemination of AMF propagules.

Macrofauna – Earthworms and termites- Earthworms (Phylum Annelida) eat detritivores (decomposing plant material) on the soil surface and in the soil, where they also eat microbiota, including mycorrhizal fungus, and speed up the mineralization of minerals like nitrogen into plant-available forms. In drier and hotter places with limited tillage, ants and termites perform comparable functions to earthworms as ecosystem engineers, and they may provide valuable ecosystem services in dryland agriculture in Mediterranean-type and arid climates (Evans et al. 2011).

Weidner et al. (2017) demonstrated that, the effect of protozoa on the activity of rhizobacteria that promote plant growth. Protozoa play an important role in soil ecosystems. Protozoa can have an impact on plant health by mineralizing nutrients and changing the structure and activity of root-associated communities. Predation by protozoa, for example, may increase the production of plant growth hormones or the survival of beneficial bacteria that suppress diseases. Furthermore, protozoa can encourage the creation of chemicals that are associated with disease suppression, such as antibiotics or siderophores. As a result, they anticipated that co-inoculating beneficial bacteria with bacterivorous protozoa would result in increased plant growth and health. They believe that interactions between plant-beneficial bacteria and protozoa may be a significant driver of their effect on plant growth in natural soil based on their research. When using helpful bacteria, native protozoa should be taken into account. Furthermore, co-inoculation of protozoa with helpful bacteria may be an effective method of increasing the plant-beneficial action of bacterial inoculants. When using helpful bacteria, native protozoa should be taken into account. Furthermore, co-inoculation of protozoa with helpful bacteria may be an effective method of increasing the plant-beneficial action of bacterial inoculants.

4 Endophytes

Just like a human body, a plant's body is a resident of many indigenous organisms, called endophytes. There are innumerable organisms living in and on a plant's body, starting from the root tips to the shoot tips without causing any infection to the host. It is a symbiotic relationship that is established between the host plant and its resident microorganisms. These microorganisms mainly involve bacteria, *Actinomycetes,* and fungi. Colonization of microorganisms in the tissues of plants is an asset, as due to symbiotic association plants grow more efficiently in the presence of the endophytes and return give space and food for endophytic living. Being resident to the host plant cells, they face very less competition from the soil microorganisms (Khan et al. 2017; Maheshwari and Annapurna 2017). When the competition reduces, it becomes easy to grow and proliferate the endophytic organisms indirectly increasing the crop yield. Endophytes are found almost in all plant species globally (Mengistu 2020). There are several types of interactions involved in this process, which were already discussed in the earlier topics. The relation of endophytes with the host plant may vary from mutualism to latent phytopathogenesis (Vyas 2018). Endophytes are of two types; obligate endophytes are strictly dependent on the host plant, whereas, facultative endophytes depend on the host plant as well as on soil, they switch depending upon the stress conditions.

In various ecological niches, plants would be found to live in a very proximal relation with the microorganisms (Omomowo and Babalola 2019). In the root system, rhizospheric organisms are found, on the leaf surfaces epiphytic organisms are present, and in the internal tissues of a plant endophytic organisms live. The rhizosphere microenvironment is the central interface to exchange resources between the plant's roots and the surrounding soil (Mendes et al. 2013). These endophytic organisms are sensitive to various biotic and abiotic factors. Temperature, humidity, wind speed, climate change, presence of organic and inorganic components in the soil and the atmosphere, presence of electrolytes, and biotic stress as per the ecological need play a very crucial need in the growth of Endophytic organisms in turn resulting in the development of the crop. Several microorganisms inhabit a seed, and a definitive structure of the seed microbiota is the aftereffect of transmission of microorganisms from the mother plant through the interior or flower pathways or from the environment (Rodríguez et al. 2018; Lamichhane et al. 2018). Microbiota present in the seed may be beneficial (plant growth promoting), inimical (plant growth inhibiting), or commensal in their interaction with the host (Lamichhane et al. 2018; Barbetti et al. 2006). Microorganisms of benefit produce many enzymes, phytohormones, several secondary metabolites (such as antibiotics), and also some antimicrobial compounds, which improves the seed germination process arising a healthy and increased yield production crop (Shahzad et al. 2018; Truyens et al. 2015).

Endophytes play a major role in the action and reaction to bioinoculants. Reports show that PGPR (Plant Growth Promoting Rhizobacteria) helps in plant growth in three ways. One is by secreting Indole-3-acetic acid (IAA) an auxin, two by increasing the availability of nutrients for plant uptake, and three by the production of antimicrobial compounds such as lytic enzymes, HCN to protect them from any kind of diseases (Ogale et al. 2018).

Other than being present on the seed, bacteria enter a plant from the rhizosphere (soil around the plant roots). Such endophytic organisms form a very intimate association due to which it brings no outside contamination or a detrimental impact on the host (Walia et al. 2017). Root apex (through a wound or auxin-induced tumor) gives a lobby to the endophytic bacteria to enter from the root hairs and spaces between epidermal cells. These organisms are arranged in microcolonies encompassing several tens and hundreds of cells. These endophytes have certain cellulolytic enzymes (such as endoglucanase, pectinase, chitinase, hemicellulose, amylase, and cellulase) which help in penetration by degrading plant's exodermal layer (Malfanova 2010). These enzymes also excite the defensive pathways in the plant when under the stress (Norman-Setterblad et al. 2000). After the bacteria gains entry it moves toward the intracellular spaces of the cortex, from where bacteria colonize in different places as endophytic organisms. Many different species and genera of an organism are observed in a single species of plants (Mehta et al. 2015; Ryan et al. 2008; Walia et al. 2017).

Endophytes promote seed germination and plant attachment under hostile conditions, additionally, they also help with the protection of heavy metals and xenobiotic compounds. Endophytes (bacteria, *Actinomycetes,* and fungi) have shown the enhancement of crop productivity and provide resistance to severe conditions such as drought or any other unfavorable soil circumstances (Taurian et al. 2010). An endophytic fungus *Pestalotiopsis neglecta* of *Cupressus torulosa*, produces flavonoids, alkaloids, phenols carbohydrates, and tannins (Sharma et al. 2016). *Actinomycetes* produce several essential secondary metabolites as therapeutic agents (Bull and Stach 2007). Endophytic bacteria such as *Streptomyces, brevibacterium, microbacterium*, and much more help in phosphate solubilization, N_2 fixation, and ethylene inhibition (Singh et al. 2017). For resisting plant invasions endophytes also synthesize hydrolases such as cellulase, proteinases, esterases, and lipases, producing antagonistic activities (Tan and Zou 2001).

5 Techniques to apply bioinoculants

Plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) are included in the bioinoculant. Endophytes and rhizobacteria that show symbiotic interaction with plants are exploited as bioinoculants in various ways (Verma et al. 2019; Singh et al. 2016). Bioinoculants are environment-friendly and provide nutrients to the host plants through direct or indirect means. The direct approaches aid in plant growth stimulation through nitrogen fixation, cytokinin synthesis, ACC Deaminase, auxin, gibberellin, and phosphorous solubilization (Olanrewaju et al. 2017). Bioinoculant suppress the growth of pathogenic bacteria through indirect mechanisms such as induced systemic resistance, siderophore synthesis, hydrogen cyanide generation, and lytic enzymes (Shukla 2020).

"Bioformulation is defined as any biologically active substances derived from microbial biomass or product containing microbes and their metabolites that could be used in plant growth promotion, nutrient acquisition, and disease control in an eco-friendly manner (Aamir et al. 2020)." Bioformulation aims to locate the ideal microbial consortia for plant growth. The use of bioformulation to improve the efficacy of bioinoculants entails combining numerous approaches. Various bio-formulations can produce a certain level of shortened-shelf life (low-quality) bioinoculants (de-Bashan et al. 2012). New bioformulation procedures with a long shelf life bioinoculant have been implemented to tackle this issue (Chaudhary et al. 2020). Bacterial strains are combined with a homogeneous combination of the carrier applied in the field when using formulation procedures (Vassilev et al. 2020) (Fig. 2).

5.1 Immersing bioinoculants in liquid suspension technique

Microbial cells are mixed in desired chemical liquid suspensions which is prepared to support growth and viability of the chosen microbial cells and even to boost adhesiveness and stability of strains (Singh et al. 2016). The liquid suspension contains nutrients, minerals and other growth promoting substances that provide favorable and optimal conditions for the growth of the microbial strains. These suspensions are prepared under controlled and sterile environments to avoid contamination. The microbial cells in the suspension helps in increasing the shelf life under adverse environmental conditions (Suman et al. 2016; Nagachandrabose 2018). The immersion of bioinoculants in liquids offers numerous advantages which include even distribution of bioinoculants ensuring the beneficial microbes are spread consistently across the target area. Liquid bioformulations enhance the viability and shelf life of microbial cells better than the dry formulations. The suspensions are easy to handle as compared to the dry formulations which require specific equipment. Better adhesion and coating on roots allowing for better establishment of microbes (Berninger et al. 2018). The liquid suspensions transition into soil rapidly facilitating quicker colonization and integration with exiting soil microbial community. The microbes are in less stressed state in liquid suspensions than in suspensions generate less dust and airborne contamination making it important for worker dry formulations which enhances their initial survival rates upon application. The scalability of liquid suspensions is better and can be used for large agricultural areas making it suitable for commercial farming applications (Alemayehu et al. 2022). These safety and environmental considerations. One of the major advantages of liquid suspension is customization of the suspension with nutrients, plant growth promoting suspensions to enhance their effectiveness at initial and later stages (Lobo et al. 2019). However, there are certain drawbacks faced in this technique such as; preservation at cold temperatures, transportation and storage, unaffordability in developing countries, and finite shelf life of microbial cells. The liquid suspensions cane be used in number of ways to deliver the beneficial microbes to the host. Seed coating is one such technique where the seeds are coated with the suspension which facilitates direct contact between beneficial micorbes and emerging roots of germinating seedlings. Soil drenching can also be performed wherein the suspensions are mixed with the soil around the root zone which helps the bioinoculants to establish themselves in the rhizosphere. Foliar spraying of the suspensions is another technique which doesn't establish microbes in the rhizosphere but can provide immediate benefits to plant foliage. These suspensions can also be used in hydroponic systems to enhance root colonization by beneficial microbes. There are certain additives added to the suspensions which include glycerol (to prevent dehydration), Polyvinylpyrrolidone (PVP),

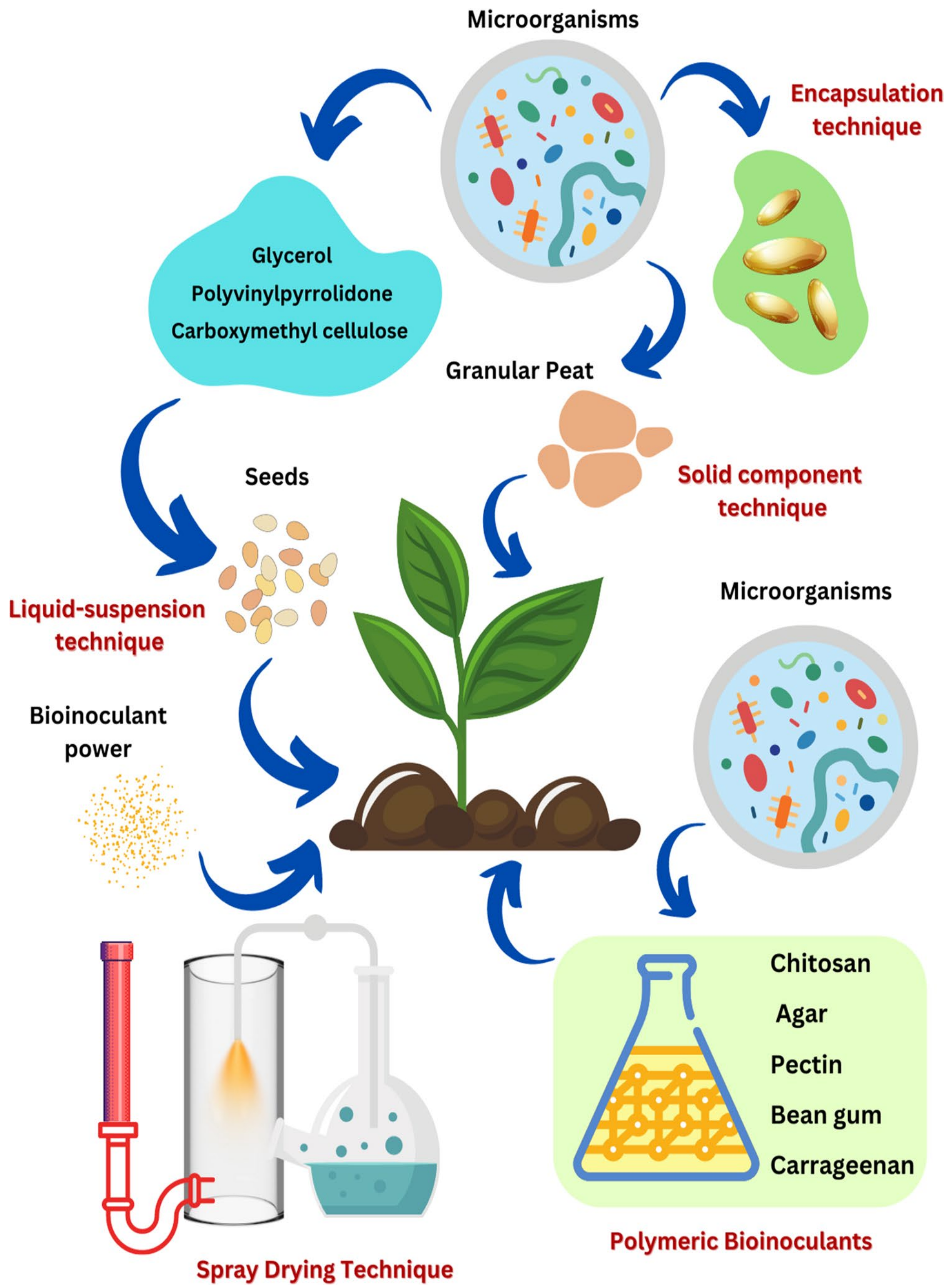


Fig. 2 Different techniques to apply bioinoculants development. The illustration describes the cutting-edge industrial techniques for the development of quality bioinoculants to increase agricultural yield

and Carboxymethyl cellulose (CMC) (Shukla 2020). In the case of CMC and PVP, various biocontrol agents, such as *Pantoea aeruginosa*, are utilized in a liquid medium enhanced with 0.1 percent CMC and sprayed on ryegrass to suppress grey leaf patches. Many bioinoculants, such as *Acinetobacter calcoaceticus, Brevibacillus brevis*, and *Micrococcus* sp., are also employed to help *Jatropha curcas* flourish under difficult conditions (Jha and Saraf 2012). PVP, like gum Arabic, is important for *rhizobial* species' survival (Shukla 2020).

5.2 Fabrication of inoculants using solid component

The most common and oldest way of formulating bioinoculants is by using solid carriers. Both forms of inoculants, organic and inorganic carriers, are used in solid carriers. Peat is the major component of inoculants that act as organic carriers in solid carriers. Peat is useful for all types of growth-promoting bacteria, although it is hard to come by in some places. Rhizobial species fit better with peat carriers than any other promoting bacterium. The quality of inoculants, pH, particle size and humidity are all important considerations throughout the formulation process (Lobo et al. 201b). The inoculation technique varies depending on the physical state of the peat like viscous liquid or granules. The first experiment was done in which peat was inoculated with *Rhizobium leguminosarum* in granular form in *Pisum sativum*, seed production was too high when compared to a liquid formulation at a moderate temperature (Dubey et al. 2019).

Granular peat is the best of all the physical forms of peat, followed by peat powder and liquid formulation (Clayton et al. 2004). However, in Australia, slurry inoculants have been shown to produce greater outcomes than granular inoculants (Denton et al. 2009). To improve the formulations of granular peat, a huge range of substances are mixed in with the microorganisms. Vermiculite, *Agaricus bisporus*, and chitin is the main constituents, which are utilized to boost microbial activity. Some fungi employed as amendments also act as biocontrol agents against a variety of diseases. For groundnut and pigeon pea crops, a combination of *Aspergillus niger* and *Fusarium udum* can result in high biomass of plant growth with positive biological control (Amer and Utkhede 2000; Singh et al. 2020).

Coal, bagasse, vermiculite, and lignite are some of the other carriers used in addition to peat (Batista and Singh 2021). However, except peat, none of these carriers can be used as a foundation for industrial application on a broad scale. Inorganic inoculants are made from synthetic and natural inorganic polymers, in addition to organic inoculants, Polymeric preparation is the most recent method of bioformulation preparation when compared to inorganic preparation. Both forms of inoculants, on the other hand, are utilized on a modest scale in agricultural techniques and are known for laboratory investigations (Shukla 2020).

5.3 The approach of polymeric bioinoculants

Synthetic carriers, as opposed to coal and peat carriers, are commonly utilized in the polymeric formulation. It has a higher cell density, a longer shelf life, and a high survival rate, all of which contribute to improved field performance (Shukla 2020). Chitosan, agar, pectin, bean gum, and carrageenan are incorporated as polymers in this recipe. These polymers are environmentally friendly and useful in agriculture. Some of the basic requirements of these polymers are that they are free of harmful preservatives that can harm both inoculated plants and bacterial cells, that they are gradually degradable by the microbial community in the soil during seedling germination and emergence, that they provide protection and act as defenders against stress conditions and soil competitors (Dangi et al. 2018), that they contain an adequate supply of water for bacteria to survive(Covarrubias et al. 2012), that they have bacterial translocation from polymer carrier to plants is facilitated by dissolving in water.

Some advantages of a potent bioinoculant include the ability to be dried and stored at room temperature for longer periods, the capacity to switch according to the needs of microbial species, the ability to provide a consistent and high-quality batch environment for microbes, and the role in improving all nutritional requirements for a higher microbe survival rate. Aside from the benefits, there are several disadvantages to employing polymeric bioinoculants, such as the handling technique and the high cost. In comparison to solid formulations, polymeric formulations are less commonly used and are now unavailable on a commercial scale because of their high cost (Shukla 2020).

5.4 Techniques involving encapsulation

Microbial cells are protected by a tiny capsule or shell in the encapsulation procedure. There are two types of encapsulation processes: micro and macro-encapsulation. Microencapsulation is a technique in which cells are encased in another substance on a small scale, often less than a hundred microns. The materials inside the capsule were released either by external force or by diffusion over time. Cells are encased in massive polymeric substances such as resins or plastics during the macro-encapsulation process. The main disadvantage of macro encapsulation is that bioinoculants are less consistent when mixed with seeds (Shukla 2020). As a result, microencapsulation loses fewer viable cells than macro-encapsulation.

Microbeads are manufactured and coated with hydrogel capsules in this method. Depending on the microbial cells and coated chemicals, the texture and shape of the beads vary (Kabir et al. 2018). With one or more microbial cells, the form can be uneven, globular, or oval. Cells in multilayered microbeads have a longer lifespan than single-layered cells. Microbial cells are more consistently combined with coated materials in the single-layered method. Synthetic and polysaccharide polymers are commonly used for encapsulating. Depending on their chemical composition, these polymers might be homo or copolymers. These monomer units have a variety of interactions, including hydrophobic interactions, hydrogen bonding, and intermolecular interactions. The use of polyethylene glycol (PEG) in the encapsulation process has decreased due to its high molecular weight (Milián et al. 2017). There are the certain method of it namely,

5.5 The approach of spray drying

Spray drying is a well-known procedure in all sorts of microencapsulation techniques since it allows for large-scale material production. However, because microorganisms have a high mortality rate, their need is diminished (Picot and Lacroix 2003). It has been discovered that extreme temperatures and dryness cause microbial cells to become inactive. In the grinding system, fine powder particles are generated by the emulsification and spray-drying processes. For a sufficient death rate of heat-susceptible bacteria, the particle size of cultures powder is reduced using the micronization process (Picot and Lacroix 2003). Broth culture can be sprayed after drying in a polymer combined with a heated compartment, according to reports (Ghosh 2006). This type of hot compartment is usually maintained closed to keep non-sporulating microorganisms like rhizobium alive. Other sporulating bacteria and fungi, on the other hand, can withstand high temperatures and dry environments.

The performance of small beads is far superior to that of large beads. However, the main disadvantage of having a pressure nozzle is the high rate of microbe mortality, which might injure the shell and cause the capsule to break down. The expense of various sorts of apparatus, as well as the high cost of carriers and power consumption, is excessive. None of the microorganisms in the microencapsulation of *P. fluorescens* by the spray drying technique were alive at 80℃, according to the findings. Despite the excellent survival rate, the bacterial colony was found to have nearly 108 CFU/g count at 60℃. The moisture content in the microcapsule was lowered when the temperature was raised from 65℃ to 80℃ for the first period. As a result, it's not surprising that dryness damages the outer protective and microbial cells (Shukla 2020). Through the spray drying process, the rate of nutrition has an impact on the survival rate of microbial cells. It was discovered that the lower the feed rate, the lower the microbial cell survival rate. There were no microbial cells present when the rate was 4–5 mL/min at first, but as the rate increased, their survival rate increased to 105 CFU/g. When the moisture content increases during spray drying, the survival rate also doubles (Amiet-Charpentier 1999).

6 Applications of bioinoculants

The application of bioinoculants leads to the advantages to the field over other techniques due to the use of living microorganisms. Detailed applications of bioinoculants are mentioned below (Table 1):

7 Discussion

For many years several approaches are experimented with for enhancing crop productivity. Many of the techniques are detrimental to the soil or harmful to the rest of the ecosystem. Some of the techniques namely; metabolite-based formulations, liquid-based formulations, solid carrier-based organic and inorganics formulations, and synthetic polymer base formulations were used (Rhizotrophs: Plant Growth Promotion to Bioremediation 2017). Systems Biology is a way to deal with complex organic frameworks through enormous scope measurement of various biomolecules. With this altering of the microbial metabolic network is possible (Chaudhary and Shukla 2018). The development of new computational techniques and modern technological approaches have changed the strategies of emerging new metabolomics and genetics to predict its metabolic networks (Baart and Martens 2012; Singh et al. 2016). Recent studies show that spread of metatranscriptomics and metagenomics in studying the microbial communal would be more relevant in rhizospheric microbial culture.

Metabolomics is a technique used to study various cell and tissue metabolites which play an essential role the microbial metabolic pathways (Burger 1963). Qualitative and quantitative measurement is made to learn the biochemical molecules of the microbes which help in a better understanding of their genetic function (Wang et al. 2013). Techniques such as chromatography, electrophoresis, and metabolite labeling are used to study proteomics of the microbial flora. During stress conditions, a gel-based protein profiling system is also used. Some of the processes such as rhizosphere colonization and adaptation due to root exudates can also be studied by transcriptomics and genomics approaches (Compant et al. 2010).

8 Challenges and future prospects

Bioinoculants are beneficial microbes with potential to make farming more sustainable. Despite having umpteen advantages there are many roadblocks which need to be addressed to engage them in broader and successful. One of the major challenges faced is that bioinoculants may not work the same wat in different situations. There are many extraneous factors like soil, weather and other indigenous organisms which can affect their activity. Another issue is the lack of standardized testing and quality control checks for bioinoculants products. Ensuring the viability and effectiveness of these strains during transportation and storage is important for their successful application in fields (Chaudhary et al. 2020). Establishing standard rules and regulations regarding the bioinoculant products will help build trust among the farmers. Cost can also be a limiting factor because making high quality bioinoculants can be expensive which may deter farmers for investing in them. Approaches to reduce production cost making it more affordable and accessible for their widespread use in agricultural systems (Mosqueda et al. 2021). A deeper understanding of the interactions between plants and bioinoculants and optimization of their applications is necessary to maximize their efficacy in the fields. In defiance of many challenges with the existing technologies advancements and research these can be overcome. The bioinoculants can be used in precision farming which involves tailoring specific formulations for different crops

Table 1 Application of bioinoculants for the growth promotion of the plants

No.	Micro organism	Inoculated plant	Effect of inoculation	References
1	*Bacillus thuringiensis*	*Fagonia mollis* and *Achillea fragrantissima*	Improved plant growth, increased water uptake, chlorophyll content and fruit yield of plant	(ALKahtani et al. 2020)
2	*Azospirillum sp.*	Rice Plant	Increased nitrogen fixation capacity of plant, phytohormone production, higher pellicle length, higher grain yield and higher straw yield	(Patel et al. 2016)
3	*Glomus mosseae*	*Erythrina variegata*	helps plant to overcome drought stress	(Al-Karaki et al. 2004)
4	Arbuscular Mycorrhizal Fungi	*Panicum turgidum*	Enhance salt tolerance	(Husaini et al. 2012)
5	endophytic fungus *P.indica*	Barley	Helps in salt tolerance and disease resistance and increased yield	(Vadassery et al. 2009)
6	*P.indica*	*Arabidopsis sp.*	Promotes growth of seedlings and helps to increase intracellular calcium elevation in roots	(Singh et al. 2013)
7	*B. subtilis*	*Lens esculenta*	Enhance efficiency of Rhizobium-Legume symbiosis	(Pandey 2009)
8	*P.corrugata*	*Amaranthus paniculatus* and *Eleusine coracana*	Increase in growth and nutrient uptake in wheat seedlings	(Pandey et al. 1999)
9	Co-inoculation of *Glomus intraradices* and *Rhizobium tropici* CIAT899	*Phaseolus vulgaris* L	Significant increase in crop yield (increase in P uptake and N fixation)	(Tajini et al. 2011)
10	Co-inoculation of *Pseudomonas striata* and *Piriformospora indica*	*Cicer arietinum* L	Synergistic effect and enhanced crop yield	(Meena et al. 2010)
11	Co-inoculation of *Piriformospora indica* and *fluorescent Pseudomonas* R62 and R81	*Vigna mungo* L	Increased growth	(Kumar et al. 2012)
12	Co-inoculation of *Piriformospora indica*, *Glomus mosseae*, and *Rhizobium*	*Vigna mungo* L	Increased growth	(Ray and Valsalakumar 2010)
13	Co-inoculation of *Azospirillium* and AM fungi	Rice plants	Enhanced growth of plant under water deficit and well-watered conditions	(Ruiz-Sánchez et al. 2010)
14	Co-inoculation of *Pseudomonas monteilii, Cronobacter dublinensis* and *Bacillus* spp	Ocimum Basilicum L	Improved oil yield	(Singh et al. 2013)
15	Co-inoculation of of *Azotobacter chroococcum* and *Azospirillium brasilense*	Maize plant	Increase in actinomycetes population for nitrogen fixation	(Pandey et al. 1998)
16	Co-inoculation of *Pseudomonas* sp. strain PGERs17 and NARs9	Wheat plant	Higher seed germination and root and shoot lengths	(Mishra et al. 2009)
17	Co-inoculation of *Pythium ultimum, P. arrhenomanes, and F. graminearum*	Maize	Disease suppression and higher growth of seedlings	(Pandey et al. 2001)
18	*Bradyrhizobium japonicum*	Soybean	Enhanced root nodule formation and yield	(Leggett et al. 2017)
19	Co-inoculation of *Bradyrhizobium, Azospirillum, Bacillus* and *Pseudomonas*	Soybean	Increased nodule number (11.40%) and biomass of nodule (6.47%), root (12.84%), and shoot (6.53%)	(Zeffa et al. 2020)
20	*Azospirillum brasilense* Ab-V5	Maize	Increased growth, yield and nitrogen use efficiency	(Zeffa et al. 2019)
21	*Rhizobium leguminosarum* sv. viciae	Pea	Enhanced nodulation and growth	(Bourion et al. 2018)
22	*Azospirillum brasilense*, *Bacillus*, and *Pseudomonas fluorescens*	Sugarcane	Increased shoot yield, P accumulation in cane, reduced P fertilization	(Rosa et al. 2020)
23	Co-inoculation of *Rhizobium meliloti*, *Paenibacillus polymyxa* and *Bacillus megaterium*	Bean	Increase in dry matter, nodule and dry root weight	(Korir et al. 2017)

and conditions helping the farmers to optimize the usage to maximize the benefits. The formulations can be applied in organic and regenerative farming practices reducing the dependence on synthetic fertilizers and pesticides Anticipating a larger uptake of these advantageous microbes as research progresses and industry standards are set, will result in more resilient and sustainable agricultural systems. These bioinoculants are the pioneers in the fight for a more sustainable and food-secure future because of their capacity to better the soil health, crop productivity, and lessen the harsh environmental effects.

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