International Journal of Recent Development in Engineering and Technology
Website: www.ijrdet.com (ISSN 2347-6435 (Online) Volume 15, Issue 03, March 2026)

From Soil to Progress: Microbial Consortia as Plant Biostimulants in Feasible Horticulture.

Kiruthika P[1], Sunitha V Hegde[2], Anu Kiruthika[3]

[1]*Assistant Professor, Department of Microbiology, Maharani Cluster University, Bangalore, India*
[2]*Assistant Professor, Department of Microbiology, Biotechnology & Food Technology, Bangalore University, Jnana Bharathi Campus, Bangalore, India*
[3]*Associate Professor, Department of Life Sciences, Indian Academy Degree College – Autonomous, Hennur Main road, Kalyan Nagar, Bangalore, India*

***Abstract*--** **As global agricultural challenges escalate and the demand for sustainable practices intensifies, the potential of microbial consortia as sustainable plant biostimulants has garnered significant attention. Microbial consortia, comprising diverse and synergistic microorganisms, offer a promising avenue to enhance plant growth, nutrient acquisition and stress tolerance, while reducing reliance on chemical inputs. The soil, a reservoir of microbial diversity, harbours an array of beneficial microorganisms capable of stimulating plant growth and health. The interactions within microbial consortia are governed by sophisticated communication mechanisms, including quorum sensing, which enables these microorganisms to communicate and coordinate their activities based on population density. Understanding the intricacies of microbial consortia's communication network provides essential insights into harnessing their full potential as biostimulants.**

***Keywords*--** **Microbial consortia, sustainable agriculture, plant biostimulants, stress tolerance, quorum sensing.**

I. INTRODUCTION

Today, there is a need to produce enough food for the more than 7 billion people on the planet and it is expected that by the year 2050, the global population will reach ~9.5 billion. Moreover, in 2020, it was estimated that approximately 900 million people were malnourished. For decades, the indiscriminate use of agrochemicals (mainly chemical fertilizers and pesticides) in agriculture to increase production and/or decrease the constant threat of infections caused by plant pathogens has led to a loss of plant health. Unfortunately, the ways in which the production of the various agricultural systems has increased in the vast majority of countries is not sustainable. Agricultural soils, directly or indirectly, are continuously losing their quality and physical properties (soil texture, permeability, porosity and drainage), as well as their chemical (imbalance of nutritive elements) and biological (beneficial organisms) health. In the case of the soil microbiota, some authors have shown that pesticides can decrease their abundance and diversity, leading to an impairment of their functioning in agro-systems.

Pesticides also negatively impact other beneficial organisms in agriculture, such as pollinating insects, which are important in improving the production of several crops. In certain regions of the world, attempts have been made to reduce or eliminate the use of potentially deleterious agrochemicals, mainly due to their risk to human health, but in many developing countries, they continue to be used without any type of regulation.

There is a pressing need for approaches that facilitate food production without the excessive use of agrochemicals and for the use of genetically improved crops, including selection of plant varieties that are resistant to pests and various adverse environmental conditions. Much of this may be achieved by the genetic modification of plants and/or the application of plant-growth-promoting microorganisms. In some cases, using a mixture of two or more compatible microorganisms of different species (or strains) can facilitate beneficial additive or synergistic results, since the lack of activities in one added microbe can be found through the action of the other. Here, it is important to define these two concepts in the context of this work. An additive effect is the sum of activities, while synergy refers to an effect that goes beyond the sum of individual actions, since there is a stimulation of one action (or microorganism) by another.

These new “plant microbiome engineering” approaches, consisting of adding effective bioinoculants, induce new structured biological networks in diverse soil types. This promotes the recovery of functional, beneficial microbial groups that are positively linked to soil fertility and replenishes the natural microbiome, which has been reduced by crop domestication practices. The addition of microbial consortia, therefore, can restructure and stimulate plant-growth-promoting mechanisms in both optimal conditions and under different types of biotic and abiotic stress. Here, the strategy of designing microbial consortia between bacteria, Trichoderma and/or arbuscular mycorrhizae fungi to stimulate plant growth is reviewed; this is a strategy that is expected to significantly increase agricultural productivity.

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Some microbial species have the ability to degrade or detoxify pollutants in the soil or water, including pesticides and organic contaminants. They break down these substances into less harmful forms, contributing to the remediation of polluted environments and reducing the negative impact on ecosystems. By harnessing the power of microbes, farmers can adopt more sustainable agricultural practices. Incorporating microbial-based products, such as biofertilizers and biopesticides, into farming systems can reduce the use of synthetic inputs, minimize environmental pollution and improve overall ecosystem health. Additionally, promoting the diversity and abundance of beneficial microbes through practices like cover cropping and reduced tillage can further enhance sustainability in farming.

II. Microbial Consortia As Biostimulants

Microbial consortia refer to a community of different microorganisms that interact with each other and their environment. In the context of agriculture, microbial consortia are gaining attention as biostimulants, which are substances or microorganisms applied to plants or the soil to enhance nutrient uptake, stress tolerance and overall plant growth. Combining fungi and bacteria together as a bio stimulant can have synergistic effects on plant growth and health. This approach often referred to as microbial consortia or mixed inoculants, takes advantage of the complementary functions and interactions between different microorganisms. Bio stimulants are substances or microorganisms that, when applied to plants or the environment, stimulate natural processes to enhance nutrient uptake, growth and stress tolerance. They differ from traditional fertilizers, which primarily provide essential nutrients to plants. Bio stimulants, on the other hand, work through various mechanisms such as enhancing nutrient availability, promoting hormone balance, stimulating root development and improving stress tolerance. Microbial consortia are designed to take advantage of the synergistic interactions among different microorganisms. The combined action of different species can result in complementary functions, such as nutrient cycling, disease suppression and plant growth. Microbial consortia are characterized by their complexity and diversity, as they encompass multiple species with different roles and functions. This diversity can provide a more comprehensive range of benefits to plants and soil ecosystems.

Several combinations of bacterial strains were tested both under in-vitro and in-vivo conditions among which the combinations of the following native bacterial isolates from Sikkim viz. *B. subtilis* BioC-WB (NAIMCC-B-02285), *B. luciferensis* K2 (NAIMCC-B02286) and *B. amyloliquefaciens* K12 (NAIMCC-B-02288) were found suitable for preparation of "Microbial formulation" for plant health improvement. Microbial consortium prepared by using indigenous microbes isolated from Sikkim soils for plant growth promotion and disease management have shown better performance for yield improvement in important different vegetable and rice crop (Panneerselvam *et al*., 2020).

III. Concept Of Using Microbial Consortia As Bio Stimulants

The concept of using microbial consortia as bio stimulants involves to curb the power of diverse microorganisms working together to enhance plant growth, health and productivity. The key points regarding the concept of using microbial consortia as bio stimulants:

3.1. Functional Diversity:

Functional diversity is increasingly recognized by microbial ecologists as the essential link between biodiversity patterns and ecosystem functioning, determining the trophic relationships and interactions between microorganisms, their participation in biogeochemical cycles and their responses to environmental changes. Microbial communities play key roles in nearly every biogeochemical process that makes earth inhabitable. They mediate vital ecosystem processes such as primary production, decomposition, nutrient cycling, climate regulation, carbon storage, disease propagation and pollutant transformation (Ducklow, 2008).

Microbial consortia consist of a combination of different microorganisms, including bacteria, fungi, archaea and other microorganisms. Each member of the consortium brings unique functions and capabilities, resulting in a broad range of benefits for plants. The functional diversity within the consortium allows for complementary interactions and synergistic effects. Microorganisms within a consortium can interact synergistically, promoting nutrient cycling, disease suppression and overall plant and soil health. For example, mycorrhizal fungi can enhance nutrient uptake, particularly phosphorus, while certain bacteria can fix atmospheric nitrogen or solubilize nutrients. These complementary interactions improve nutrient availability and utilization by plants.

3.2. Enhanced Plant Growth:

Microbial consortia promote plant growth through various mechanisms. Plant-growth-promoting microorganism (PGPM) (e.g., bacteria, actinomycetes, fungi and algae) that have a beneficial effect on plant growth through the action of either direct or indirect mechanisms (Abhilash *et al*., 2016). They can produce plant growth-promoting substances, such as phytohormones or enzymes that stimulate root development, nutrient uptake and overall plant vigor. The combination of microorganisms within the consortium can provide a more comprehensive and effective approach to plant growth promotion.

3.3. Disease Suppression:

Microbial consortia can contribute to disease suppression by employing multiple mechanisms. Different microorganisms within the consortium may produce antimicrobial compounds, compete with pathogens for resources, or induce systemic resistance in plants. The presence of multiple microorganisms with different modes of action increases the chances of effective disease control. Some microbial consortia have the ability to suppress plant pathogens through various mechanisms, such as the production of antimicrobial compounds or competition for resources. This can reduce the need for chemical pesticides.

3.4. Soil Health and Function:

Mixed inoculants positively influence soil health and function. They contribute to organic matter decomposition, nutrient cycling, soil aggregation and improvement of soil structure. The presence of diverse microorganisms within the consortium enhances microbial diversity and activity, leading to a more balanced and resilient soil ecosystem. The activities of microbial consortia can improve soil structure, water retention and overall soil health. Healthy soil with an active microbial community is essential for sustainable agriculture.

3.5 Environmental Adaptability:

Microbial consortia can be tailored to specific environmental conditions, such as high salinity, drought, or contaminated soils. By including microorganisms adapted to these conditions, microbial consortia can help plants better withstand and adapt to challenging growing conditions. The interactions between different microorganisms within a consortium can lead to synergistic effects. For example, certain bacteria may enhance the growth of specific fungi and vice versa. This synergy can result in improved plant performance and resilience.

IV. Methods Involved For The Application Of Microbial Consortia In Agriculture

The application of Microbial Consortia in agriculture involves various methods to introduce beneficial microorganisms to plants, seeds, or the surrounding soil. Here are some common methods for applying microbial Consortium in agriculture:

4.1. Seed treatment:

Microorganisms can be applied directly to seeds before planting. This can be done by coating the seeds with a microbial formulation or by soaking the seeds in a probiotic suspension. The probiotics adhere to the seed surface and can colonize the emerging roots, establishing a beneficial microbial community.

4.2. Soil drench:

Microbes can be applied as a liquid solution or suspension directly to the soil around the plant roots. This method allows the probiotics to colonize the rhizosphere and interact with the plant roots, promoting growth and providing other benefits.

4.3. Foliar spray:

Microbes can be applied as a spray directly onto the foliage of plants. This method allows the probiotics to establish a presence on the leaf surfaces and can promote beneficial interactions with the plant, such as disease suppression or stress tolerance.

4.4. Root dip or transplant solution:

Microbial consortia can be applied to the roots of transplants or seedlings before they are planted in the field. This can be done by immersing the roots in a probiotic suspension or solution. The probiotics adhere to the root surfaces and can facilitate early establishment and growth of the transplants.

4.5. Irrigation or fertigation:

Microbial mixed inoculants can be applied through irrigation systems, either as a suspension or by incorporating them into the irrigation water. This method allows widespread distribution of probiotics throughout the field or greenhouse, promoting their colonization in the soil and interaction with plant roots.

4.6. Compost or organic matter incorporation:

Microbes can be introduced into compost or organic matter used for soil amendment.

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As the compost or organic matter is incorporated into the soil, it carries the beneficiary organisms, which can then establish a presence in the rhizosphere and contribute to soil health and plant growth (Joyce *et al.*, 2023).

It's important to note that the effectiveness of Microbial Consortia application methods may vary depending on the specific strains used in it, the target crops, environmental conditions and the overall management practices.

Additionally, application timing and frequency may also be important factors to consider for optimal results. Conducting field trials and monitoring plant responses are essential to determine the most effective application methods for a particular agricultural system. The use of microbial consortia as bio stimulants is an active area of research and development. Optimizing consortium composition, understanding microbial interactions and exploring their applications in different agricultural systems are ongoing efforts to maximize their benefits.

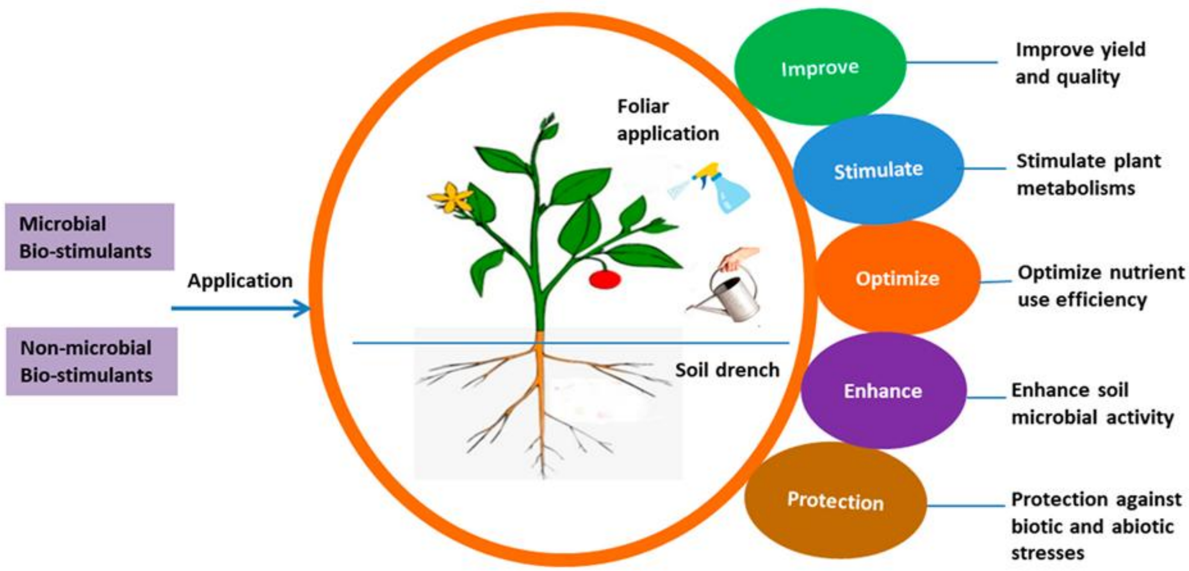

Fig 1. Application of Microbial Biostimulants and their effect on plants (Rajput *et al.*, 2019)

V. Biostimulants With Examples

Biostimulants (BSts) are organic inputs applied to plants to enhance nutrition efficiency, abiotic stress tolerance and crop quality traits, regardless of their nutrient content. Based on their origin, BSts are categorized into two broad groups: non–microbial BSts (chitosan, humic and fulvic acids, protein hydrolysates, phosphites, seaweed extracts and silicon) and microbial BSts (arbuscular mycorrhizal fungi, plant growth–promoting rhizobacteria and *Trichoderma* spp.). Microbial Consortia, when used as bio stimulants in agriculture, involve the application of beneficial microorganisms to enhance plant growth, nutrient uptake, stress tolerance and overall plant health.

Here are a few examples of microbes used as bio stimulants:

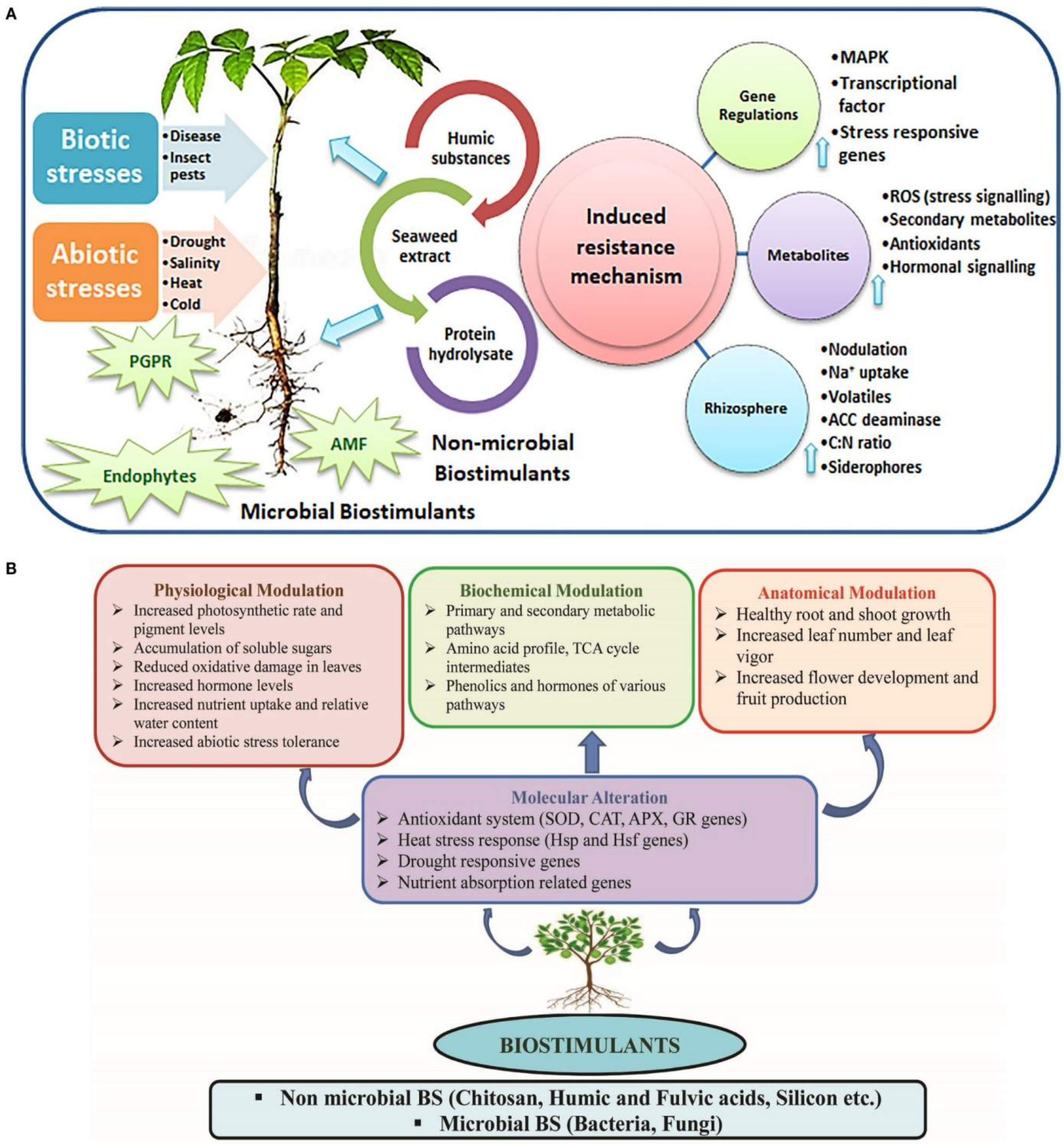

Fig. 2 (A , B) Role of Bio stimulants (Bhupenchandra *et al*., 2022)

5.1. Plant Growth-Promoting Rhizobacteria (PGPR):

Nowadays, application of PGPR consortium (two or more microbes together) as microbial biostimulants is gaining attention worldwide due to the multifaceted advantages these microbial consortia can offer for improving plant growth, yield and crop quality. PGPR are beneficial bacteria that colonize the rhizosphere (the soil surrounding plant roots) and enhance plant growth through various mechanisms.

For example, species of *Bacillus, Azotobacter, Enterobacter, Azospirillum, Xanthomonas, Cellulomonas* and *Pseudomonas* are commonly used as PGPR. PGPR release substances such as siderophores, antibiotics, pigments, organic acids (malic, acetic, citric, oxalic, lactic, formic, gluconic and 2-keto-gluconic), water-soluble vitamins (niacin, thiamine and biotin molecules or antioxidants) and volatile organic compounds (monoterpene alcohols).

International Journal of Recent Development in Engineering and Technology
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The interactions between the plant and PGPR are synergistic, leading to relevant benefits for both the plant crops and the plant microbiome. They can stimulate plant growth by fixing atmospheric nitrogen, solubilizing phosphates, producing plant growth hormones (such as auxins and cytokines) and suppressing plant pathogens. PGPR also promote the synthesis of hydrolytic enzymes (such as glucanases and chitinases) that produce morphological changes in the fungal mycelium, such as the fracture and lysis of the spikes, preventing the development of pathogenic fungi.

The selection of these strains is dependent upon the source of the strain isolation, since consortium members need to proliferate in the environmental conditions (soil type, climate and host) where they will be applied. In addition, it is important to note that, when two or more strains are part of a bacterial consortium, each strain not only competes functionally with the others for plant growth promotion, but also complements the others for soil and/or plant establishment.

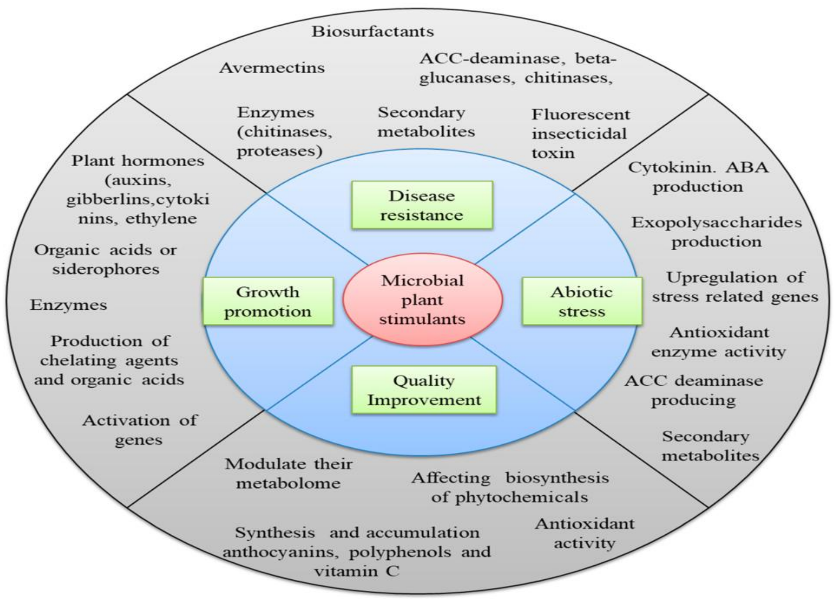

Fig 3: PGPR- Mode of Action on the growth of crop plants (Hamid *et al*., 2021)

5.2. Mycorrhizal Fungi:

Microorganisms acting as biostimulants mainly belong to beneficial fungi groups including arbuscular mycorrhizal fungi and free-living bacteria (Berg, 2009). Mycorrhizal fungi help plants in adaptation of plants to unfavourable Conditions. Mycorrhizal fungi form a symbiotic association with plant roots, facilitating nutrient uptake, particularly phosphorus. They extend the root system by forming a network of fungal hyphae, increasing the surface area available for nutrient absorption. Examples include species of *Glomus, Rhizophagus* and *Trichoderma* Mycorrhizal fungi can be applied to plant systems to establish or enhance mycorrhizal associations. These applications can be particularly beneficial in degraded or disturbed soils, agricultural settings and reforestation efforts.

5.3. Trichoderma spp.:

Trichoderma is a genus of beneficial fungi that can promote plant growth and protect against pathogens. They can colonize plant roots, enhance nutrient uptake, produce antifungal compounds and induce systemic resistance in plants. Several *Trichoderma* species have been studied for their plant growth-promoting properties, including: *T. harzianum, T.viride, T. atroviride, T.virens*. These beneficial microbes produce secondary metabolites, natural bioactive substances which are currently being evaluated as potential biopesticides. Natural compounds from microbial or other sources, able to function as plant stimulants or protectors, can be applied in combination with beneficial microbes for more efficient and wide-ranging agricultural formulations.

Trichoderma has also been used as a biostimulant because it can promote plant growth and development through its synthesis of phytohormone-like molecules and volatile organic compounds, thus improving soil mineral solubilization, nutrient uptake and translocation, increasing root system development. The combination of PGPB, mycorrhizal fungi and biocontrol agents like *Trichoderma* spp. provides a strategy that should be commercially exploited in order to endow plants with a complete "benefits package": increases in plant biomass and yield production, resistance to abiotic stresses, biocontrol of phytopathogens and better nutrient uptake (Santoyo *et al*., 2021).

5.4. Yeasts:

Use of yeast in the industry is well known, but the implementation in agriculture is not very common. Certain yeasts, such as *Saccharomyces cerevisiae, Candida* spp. & *Pichia* spp. have been used as bio stimulants. They can enhance plant growth by improving nutrient availability, stimulating root development and activating plant defense responses against pathogens. Yeast contains a large chromosome that will have a lot of functional genes that may help in pesticide degradation and induce plant growth by secretions of growth-regulating hormones. Till now, the study shows that plant growth induced either direct and/or indirect mechanisms by the bio-agents. Some literature indicates that yeast has these two properties which help to promote the plant growth attributes. It has been reported that yeast produces indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA). These compounds are major plant growth hormones produced by endophytic yeast (*Williopsis saturninus*) isolated from the root of maize (Nassar *et al*., 2005).

5.5. Cyanobacteria:

Cyanobacteria are ubiquitous in nature and their presence has long been reported in different soils (agriculture soils, rice fields, mines, desert lands, marshy soils) where they are responsible for bringing positive effects in different ecological situations (Thangaraj *et al*., 2017). Some nitrogen-fixing cyanobacteria, such as *Anabaena* spp. and *Nostoc* spp., have been explored as bio stimulants. They can fix atmospheric nitrogen, making it available to plants and contribute to soil fertility. Cyanobacteria can contribute to soil health and fertility through their activities. They can improve soil structure by producing extracellular polysaccharides that help bind soil particles together, enhancing soil aggregation and water retention capacity. Additionally, cyanobacteria can secrete enzymes that break down organic matter, contributing to nutrient cycling and organic matter decomposition in the soil.

The use of cyanobacteria as bio stimulants can have environmental advantages. By reducing the reliance on synthetic fertilizers, cyanobacteria can potentially decrease nutrient runoff into water bodies, mitigating water pollution issues associated with excessive nutrient inputs. Furthermore, cyanobacteria's carbon fixation capability contributes to carbon sequestration, potentially helping to mitigate climate change.

5.6. Lactic Acid Bacteria (LAB):

Lactic acid bacteria (LAB) have gained attention for their potential as bio stimulants in agricultural practices. LABs are commonly used as probiotics in the food industry and they can also be beneficial for plants. LAB can improve nutrient availability, enhance soil structure, suppress pathogens and stimulate plant growth. LAB can produce antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocins, which inhibit the growth of pathogens. LAB produce a diversity of antimicrobial compounds including antifungal diketopiperazines, hydroxy derivatives of fatty acids, 3- phenyllactate; antibacterial bacteriocins and bacteriocin-like compounds; and general antimicrobials such as organic acids, hydrogen peroxide, pyrrolidone-5-carboxylic acid, diacetyl and reuterin (b-OH-propionic aldehyde). It also synthesis osmoprotectants like proline and trehalose, which help plants cope with water stress. *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus brevis*, *Lactobacillus rhamnosus* and *Pediococcus acidilactici* are examples of LAB used as bio stimulants.

5.7. Penicillium spp:

Penicillium species, specifically certain strains, have been studied for their potential as biostimulants in agriculture. *Penicillium* can release organic acids and enzymes that help solubilize nutrients such as phosphorus, making them more available for plant uptake. It can produce growth-promoting substances like indole-3-acetic acid (IAA) and siderophores, which stimulate plant growth and development. *Penicillium* can activate the plant's defense responses, priming it to better defend against pathogen attacks. The resistance to insects and other organism happens when crops are expose to *Penicillium* species. It generates a type of resistance to pathogens, which is known as a Systematic Acquired Resistance (SAR), when it happens the crop activates different enzymes (Chitinases, Glucanases, Peroxidases, Oxidases and Lyases), metabolites (Salicylic and Jasmonic Acids) and phenolic compounds as a defense mechanism which protect them from attacks of pathogenic organisms (Koike *et al.,* 2001).

International Journal of Recent Development in Engineering and Technology
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This friendly fungus can wrap itself around the root & prevent other less helpful organisms from living there. It has the first chance to use the plants by products. This will make the microbe stronger and able to convert more phosphate for the roots to use. With additional phosphate, the plants will be stronger and more productive. It also breaks down organic matter, releasing nutrients and improving nutrient cycling in the soil. Examples of Penicillium Species are *Penicillium bilaiae*, *Penicillium chrysogenum, Penicillium simplicissimum.*

VI. Microbial Consortia's Communication Network - Decoding Quorum Sensing

Microbial consortia, composed of diverse microorganisms living in close proximity, engage in sophisticated communication and cooperation to thrive in complex environments. Within these communities, quorum sensing serves as a remarkable communication network, allowing microorganisms to coordinate their activities based on population density. Decoding quorum sensing has become a fascinating area of research, shedding light on the intricate mechanisms by which these microbial communities achieve harmonious interactions.

Microbes in a population continuously produce and release small signaling molecules called auto inducers into their surroundings. As the microbial population grows and reaches a certain cell density, the concentration of signaling molecules in the environment increases proportionally. Individual microbial cells have receptors on their surfaces that can detect the concentration of signaling molecules in their immediate environment. When the concentration of signaling molecules surpasses a critical threshold (the quorum), the receptors on the microbial cells are triggered. This, in turn, initiates specific intracellular signaling pathways. The activated intracellular signaling pathways influence the expression of certain genes in the microbial cells. These genes may code for various products, such as enzymes, toxins, or biofilm-forming proteins. The coordinated expression of genes across the microbial population leads to the emergence of collective behaviors and activities that benefit the entire community. Examples include: Biofilm Formation, Synchronized Virulence and Resource Utilization. The signaling molecules of QS used by most Gram-negative and Gram-positive bacteria are N-acyl homoserine lactones (AHL) and modified oligopeptides (autoinducing peptides, AIP), respectively. To date, various small cyclic furanone compounds (autoinducers-2, AI-2), widely distributed among both Gram-positive and Gram-negative bacteria, are also known, which provide intra- and interspecies communication between them (Abbamondi, 2022).

QS has proved to be a powerful tool for metabolic engineering an studies have established a foundation for the use of quorum sensing in microbial consortia.

VII. Conclusion

Microbial biostimulants are a viable alternative for supporting plants exposed to abiotic stresses in the current context of fast-developing climate change (Santoyo *et al*., 2021b). In agriculture, consortia members should positively interact, where mutualistic growth is desirable for stable performance over prolonged cultivation to obtain the expected positive effect when applied to a crop (Mahmud *et al*., 2020). In this regard, bacterial communication is only superficially understood at the present time. This communication relies on the production, detection and response to extracellular signalling molecules that regulate and shape the bacterial population in the consortium, where only compatible microbes are involved in altering the plant defense response affecting overall plant health and growth. Consortium communication is highly dependent on molecular signals; among them, quorum sensing plays a significant role in bacterial compatibility in consortium formulations. Among several signal molecules, the acyl homoserine lactone (AHL) signal molecules are the most well known in bacteria. Pure AHLs have shown induction of intracellular Ca^{2+}levels and primary root growth, while AHLs produced by PGPB, such as *Serratia liquefaciens* and *S. phymuthica*, have stimulated root development and total plant biomass; other bacteria, like *Sinorhizobium fredii* and *Pantoea ananatis*, stimulated the formation of biofilm in the roots of *Oryza sativa* (rice) and *Phaseolus vulgaris* (bean) plants. The application of microbial biostimulants might provide a long-term and cost-effective solution to plant productivity losses caused by changing climatic factors, as well as aid in the optimization of human inputs in agro-ecosystem. Future research should focus on developing better-targeted products, such as delving deeper into interactions of the microbial biostimulant with indigenous plant-associated microbiomes (Fadiji, 2022). Incorporating quorum sensing and microbial consortia in agricultural practices aligns with the principles of sustainable agriculture. By promoting beneficial interactions among microorganisms and between microorganisms and plants, farmers can potentially reduce the use of chemical inputs, increase crop resilience and improve overall agricultural productivity while minimizing environmental impacts. It's worth noting that while the potential of quorum sensing and microbial consortia in agriculture is promising, their practical implementation may vary depending on specific crop types, soil conditions and environmental factors.

Ongoing research and field trials are crucial to better understand and optimize these microbial-based approaches for sustainable agriculture.

Acknowledgment

I would like to express my sincere gratitude to Indian Academy Degree College – Autonomous and Maharani Cluster University, Bengaluru for their invaluable contributions and support in the preparation of this review paper.

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