Sukhminderjit Kaur
Vagish Dwibedi
Pramod Kumar Sahu *Editors*

Metabolomics, Proteomics and Gene Editing Approaches in Biofertilizer Industry

Volume II

Springer

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Preface

Biofertilizers refers to the live or latent microbial inoculants capable of enhancing plant nutrition and growth through various direct and indirect mechanisms. The past few decades have seen biofertilizers gaining immense ground as crop productivity faces great challenges due to ever-increasing populations and environmental threats. Researchers are looking for alternatives to enhance crop production through sustainable approaches. This book is an attempt to consolidate recent advancements related to biofertilizers onto a single platform.

The venture of the book, entitled *Metabolomics, Proteomics and Gene Editing Approaches in Biofertilizer Industry*, second volume, is to present details of cutting-edge research in the field of biofertilizers and plant-microbe interactions that will help readers understand how microbes play a significant role as biofertilizers in boosting plant production in limited inorganic nutrients. It covers general strategies for harnessing integrated technologies: omics, proteomics, and metabolomics for the development of potential biofertilizers. New techniques for enhancing the efficacy and quality of biofertilizers have been elaborated in this book through different chapters contributed by experts in the field of agricultural microbiology.

The process of gene editing tools for engineering beneficial microorganisms in biofertilizers and the role of synthetic biology to improve plant growth and resistance to stress through the use of genetic engineering techniques are also elaborated in the second volume. This volume also gives insights into the role of bioinformatics strategies for analyzing metabolomics, proteomics, and gene editing data and the role of machine learning and intellectual property rights in the biofertilizer industry. The information in this book regarding the role of different microorganisms and the applications of nanoformulations used for nutrient management will be helpful for designing next-generation bio-formulations to assist plant growth.

We believe this book will help provide a substantial number of pieces of evidence that underline the genomic basis of nutrient management by microbes. Essential information will be provided regarding the genomic and metabolomic background of biofertilizers. This collective work is distinct because of our focus on diverse emerging technologies which are high-throughput, scalable, and applicable to different countries regardless of their socio-economic conditions. We consider this a sincere effort to highlight the underutilized potential in advanced technologies in the abatement of dynamic issues in sustainable biofertilizers. This book will improve the current state of knowledge and should inspire researchers and innovators to advance the current interdisciplinary knowledge into technologies that are readily available and effectively minimize hazards associated with chemical fertilizers.

Biofertilizers are the key to sustainable agriculture, and it has become the foremost priority of plant biologists to mine soil microbial diversity with respect to their spatiotemporal, biochemical, and physiological structure and their beneficial effects on plants through modern techniques like metagenomics, proteomics, metabolomics, and the like. Hence, the knowledge in these areas is vast and often does not reach every hand. An edited book like the present one provides updated knowledge to a researcher, and it is equally challenging to compile different studies in a book. First of all, we would like to acknowledge all the contributing authors of chapters in this book for bringing such an exhaustive compilation to a wider readership. All the editors gratefully acknowledge their parent organization for all the support and encouragement rendered. Thanks are also due to all those researchers whose original work has formed the basis of this compilation; without the endless efforts of researchers, science and society could not progress. We are also thankful to the team at Springer for bringing this compilation to the scientific fraternity.

Mohali, Chandigarh, India Sukhminderjit Kaur
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Contents

1 Biofertilizers: An Imminent Approach for Sustainable Agriculture 1
Seema Garcha and Samredhi

2 Futuristic Approaches in Biofertilizer Industry: Challenges, Opportunities, and Future Directions 15
Hardeep Kaur, Shinar Athwal, and Kashish Garg

3 Metabolomics: A Paradigm Shift in Understanding Biofertilizers Dynamics 35
Nitu Rani and Narashans Alok Sagar

4 Metabolite-based Bioformulation: Next Generation of Biofertilizers 53
Richa

5 Gene Editing Tools for Engineering Beneficial Microorganism in Biofertilizer 83
T. Kavya, Haralakal Keerthi Kumari, Geetha Singh, V. Govindasamy, D. Vijaysri, J. Sushmitha, Konderu Niteesh Varma, G. R. Sneha, T. Chethan, and Sunil Jadhav

6 Methods from the Field of Synthetic Biology that Aim to Improve Plant Growth and Resistance to Stress through the Use of Genetic Engineering 99
K. Jagadeesh Chandra Bose, Sukhminderjit Kaur, Sonia Sharma, Jyoti Sarwan, and Nazim Uddin

7 From Data to Discoveries: Bioinformatics Strategies for Analyzing Metabolomics, Proteomics, and Gene Editing Data 123
Palak Rana, Gursharan Kaur, Harleen Kaur Walia, Gurleen Kaur Sodhi, Swapnil Kajale, and Vagish Dwibedi

8 The Role of Machine Learning in Biofertilizer Industry: From Data Analytics to Predictive Modelling 141
Gursharan Kaur, Palak Rana, Harleen Kaur Walia, and Vagish Dwibedi

9 The Role of Artificial Intelligence in Biofertilizer Development 157
Babita Thakur and Sukhminderjit Kaur

10 Algal Nanobiofertilizers: Prospects and Challenges 177
K. Sowmiya, K. Praveen, S. Krishna Kumar, and M. Priya

11 Nanobiofertilizers: A New Paradigm for Sustainable Agriculture. . . . 201
Yashika Yadav, Babita Thakur, and Sukhminderjit Kaur

12 Foliar-Based Nanoformulations: Leads and Flaws 223
Gajendra B. Singh, Adarsh Sharma, Joythi Thapa, and Nidhi

13 Supplementing Micronutrients to Plants Through Microbes. 247
Yalavarthi Nagaraju, Duppala Manoj Kumar, Praveen S. Patted, Pramod Kumar Sahu, and Savani Ajit Kumar

14 Co-shaping and Co-evolution of Microbial Biodiversity: Study for Identification of Potential Plant Growth Promoting Microbes. . . . 261
Shanu Magotra, Neelam Prabha Negi, and Hemant Kumar

15 Lactic Acid Bacteria for Safe and Sustainable Agriculture 283
Mai N. Amer, Maissara M. K. Elmaghraby, Aya A. M. Abdellatif, and Ibrahim M. K. Elmaghraby

16 Transforming Roles of Actinobacteria in Sustainable Agriculture: From Soil Health and Plant Productivity Perspective. 299
Aya A. M. Abdellatif, Doha A. S. Gebily, Maissara M. K. Elmaghraby, Pramod Kumar Sahu, Babita Thakur, and Sukhminderjit Kaur

17 Commercialization and Market Perspectives of Biofertilizers Through Advanced Approaches. 339
Deepak Kumar, Ritik Kumar, Sakshi, Deepshikha, and Anil Kumar

18 Intellectual Property Rights in Biofertilizer Industry. 359
Priyanka Verma and Sakshi Manhas

Editors and Contributors

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Sukhminderjit Kaur is working as Professor in the Department of Biotechnology, Chandigarh University, Mohali, India. She received her doctorate in 2008 from PGIMER, Chandigarh, India. She has experience of more than 20 years in academics and research and has published more than 58 research papers and various book chapters (16). Two patents have been granted to her credit for biofertilizer formulation. She has edited various books also with reputed publishers. She has guided four doctorate students. She is a member of numerous scientific/professional societies including Indian Science Congress, Association of Microbiologists of India, and Gastrointestinal Society of India. Her work is inclined toward exploring microorganisms in various applications such as biofertilizers development using different consortium of plant growth promoting bacteria, probiotics, and bioremediation.

Vagish Dwibedi obtained his Ph.D. from Thapar Institute of Engineering and Technology, Patiala, India. He has conducted research projects and consultancy work in plant-microbe interaction, bioassay-guided drug discovery and development, sustainable agriculture, and wastewater treatment. Presently, he is working as a visiting postdoctoral scientist at the Agriculture Research Organization, Ministry of Israel (ARO), Rishon Lezhion, Israel. Previously, he served for 2 years as an Assistant Professor in the Department of Biotechnology, Chandigarh University, Gharuan Mohali, Punjab. Formerly, he worked as Research Scientist at AGPHARM BIOINNOVATIONS LLP, incubated at Thapar Institute of Engineering and Technology, Patiala, India. Dr. Dwibedi works toward developing screening platforms for different biological activities such as antimicrobial, antioxidant, anticancer, or finding novel molecules that interfere in the mechanism of development of diseases such as Alzheimer's dementia, Parkinson's disease, obesity, anti-gout (arthritis), and type 2 diabetes.

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Biofertilizers: An Imminent Approach for Sustainable Agriculture

1

Seema Garcha and Samredhi

Abstract

In the current scenario of ever-increasing population, the demand for a sustainable agriculture supply is evident to combat food crisis. Excessive use of synthetic fertilizers has taken a toil on human and environment health putting the ecosystem in a state of turmoil. To abate the situation, biofertilizers are an excellent choice for sustainable farming. The use of plant growth promoting microorganisms has its own benefits when it comes to soil and plant health and enhanced crop yield. But challenges to use biofertilizers include the lack of sufficient availability of the product and lack of awareness in regard to concentration, time, and method of application. Also, public investments in bio-inputs are substantially lower compared to the significant subsidy provided for the chemical inputs. Central government initiatives like NITI Aayog, Paramparagat Krishi Vikas Yojana (PKVY), Mission Organic Value Chain Development for North Eastern Region (MOVCDNER) and National Food Security Mission (NFSM) provide financial assistance and subsidies to predispose the interest of farmers towards organic farming. Under the Soil Health Card Program, the Government of India has also been pushing Integrated Nutrient Management (INM) for the prudent use of pesticides to limit excessive use of fertilizers and pesticides. Conventional biofertilizer formulation limit the shelf life and efficacy of biofertilizers whereas advanced techniques like pelletized formulation using carrier material like compost and biochar are useful in improving the shelf life and efficacy of the biofertilizer. Other than this, encapsulation is another way to further increase shelf life using alginate-based microcapsules of biofertilizers which assist in boosting the cell resistance against unfavorable temperatures and pH. Additives such as glyc-

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erol, chitin, peat, and skim milk are also providing promising results influencing the cell viability and enhanced growth for a better field performance.

Keywords
Biofertilizer · Central policies · Bioformulation · Encapsulation · Additives

1.1 Introduction

Escalating human population is putting a lot of pressure on agricultural lands to produce enormous amount of food. To combat this food crisis, farmers resort to chemical means to effectively generate agricultural productivity. Though the results are quick and effective but this long-term use of chemical fertilizers has resulted in soil degradation, environmental pollution, and deterioration of human health and putting the ecosystem in state of a turmoil. In conventional farming systems, synthetic NPK fertilizers are used extensively to meet plant nutritional requirements and increase agricultural output. But only a limited dose of fertilizer which is around 30–40% actually gets utilized by the crop leaving the rest in the soil and to contributing in polluting the environment. Furthermore, chemical fertilizers contain toxic metals and radionuclides which are not easy to degrade, making them persistent contaminants in the nature. Eutrophication of water sources is another major issue associated with the use of excessive chemical fertilizers (Kumar et al. 2022). Soil microbial community is an important aspect in ecosystem for soil nutrient cycling and nutrient uptake. Long-term chemical fertilization has affected the soil fertility and microbiota showcasing diminished presence of beneficial microbes like proteobacteria which were found to be more prevalent in naturally cultivated rhizospheric zone of walnut trees than the chemically cultivated ones (Bai et al. 2020).

Excessive applications of potassium fertilizers in the soil have disrupted the delicate balance of nutrients by plants leading to reduced productivity and crop losses. Another threat to the environment posed by chemical fertilizers includes the emission of greenhouse gases (GHG) altering the global warming potential (GWP). Synthetic fertilizers stimulate soil acidification, posing a risk to underground water, weakening of plants roots and making them more susceptible to phytopathogens (Randive et al. 2021). This indiscriminate use of synthetic fertilizers destroys the key fundamentals of agricultural production jeopardizing long-term food security. Ecology suffers when nutrient ratios are out of equilibrium due to the excessive use of highly concentrated mineral fertilizers. Currently, the primary issue in agriculture is to prevent further declines in crop yield and soil health (Bhatt et al. 2019).

1.2 Solution to the Problem—Biofertilizers

Plant rhizospheric zone contains crucial microbial communities such as bacteria, archea, fungi, oocytes, nematodes, and viruses. While some maybe pathogenic, a variety among them are plant growth promoting microbes responsible for protecting the plant against biotic and abiotic stress and providing improved crop yield and productivity. Using the beneficial microbial communities as biofertilizers in sustainable farming is a novel approach for agricultural production. These are live bacterial or fungal formulations used for enhancing plant and soil health (Suhag 2016). Biofertilizers aid in enhancing plant biomass, mineral content (potassium, phosphorus, iron), shoot and root length, seed germination, and atmospheric nitrogen fixation (Glick and Gamalero 2021). Utilizing biofertilizers lowers the high cost of synthetic purchases meeting the global demand for environment friendly crop production. Also, biofertilizers supply plenty micronutrients, organic matter, phytohormone secretion along with mitigating the detrimental effects of chemical fertilizers (Mahanty et al. 2017). Admist the blatant use of inorganic fertilizers, abiotic stress on crops is also amplified by climatic variability which further lowers agricultural output. Numerous abiotic and biotic stress factors, such as drought, soil salinity, temperature fluctuations, water retention capacity, heavy metal toxication, and various plant pathogens reduce the crop yield and quality. Biofertilizers provide nutrition through natural processes such as Zn, K, and P solubilization, siderophore production, nitrogen fixation, and hydrolytic enzymes to protect the plant against stress conditions (Chaudhary et al. 2022).

Plant diseases in agriculture are conventionally controlled by the use of chemical pesticides and fungicides. Haphazard use of these pesticides is toxic to environment. Beneficial microbes are being explored in this facet as biopesticides and certain microbial strains for use as biofertilizer formulations to protect the plant against phytopathogens. Effective microbes to be studied against pesticide toxicity include *Azotobacter*, *Enterobacter*, *Paenibacillus*, *Pseudomonas*, and *Bacillus* (Shahid et al. 2019). Microbial strains which are antagonistic against variety of phytopathogens responsible for plant diseases such as root rot, necrosis, spotting, wilting, canker, etc., are recognized for their valuable contribution as biological control agents. Moreover, formulations constituting microbial consortium where combined use of more than one strains exhibit multiple mechanisms for biocontrol activity to fight against complex plant diseases. Moreover, studies reveal that consortium boosts the transcriptional activation of various metabolic pathways, which then have a significant cascading effect on the host plant's signaling for defense against various plant pathogens and some arthropod pests (Dhir 2017).

Application of *P. fuorescens* strain in Cameroon's acidic soils boosted shoot length, grain yield, plant dry weight, and seed phosphorus content in maize (Nosheen et al. 2021). When *Bacillus thuringiensis* was applied as a biofertilizer on *Abelmoschus esculentus* plants in the field, it significantly improved shoot and root length, seed germination, fruit weight, leaf diameter, seed weight as well as the total dry and fresh weight in the treated plants as compared to the non-treated ones (Bandopadhyay 2020). Integrated biofertilizer application of *Bacillus amyloliquefacien*, *Nostocmucorum*, and *Saccharomyces cerevisiae* on Cowpea (*Vigna unguiculata*) was assessed for enzymatic activities, plant growth, and crop yield. Treated plants showed significant increase in dehydrogenase enzyme activity, chlorophyll content, plant dry weight, pod length, grain yield, and vascular bundle length (Omer et al. 2023). In field studies, cyanobacterial isolates positively influenced plant health and rice productivity. Within 4 months, substantial increase in plant length was observed. Plants treated with the bioinoculant were healthier and greener than the untreated control. Panicles gathered at the ripening stage were observed for the weight and number of grains per panicle and the results recorded a significant increase in both the parameters (Iniesta-Pallarés et al. 2021). Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plants thereby acting as bio-ameliorators which positively impact the rhizospheric characteristics of soil as well as protecting the plant during stress conditions like drought and heat. Results available show that AMF plays a very important role in enhancing the nutrient uptake and stimulating the production of phytohormones like gibberellins and auxins (Suhag 2016). In leguminous plants, *Rhizobium* in the root nodule fixes atmospheric nitrogen. The plant utilizes nitrogen to synthesize amino acids, vitamins, and nucleic acids. The use of rhizobia in nitrogen fixation reduces the dependency of leguminous plants on synthetic N fertilizers (Fasusi et al. 2021).

Biofertilizers are classified into several categories based on their purpose and mode of action. Nitrogen fixing, potassium solubilizing, phosphorus solubilizing, and plant growth promoting rhizobacteria (PGPR) are the most commonly used of them all. These include *Bacillus*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, *Azospirillum*, cynobacteria, and mycorrhiza (Bhavya and Geetha 2021). For instance, nitrogen fixing biofertilizers classified as free living, symbiotic, and associative. These include *Azotobacter*, *Clostridium*, *Anabaena*, *Nostoc*, etc.

1.2.1 Classification of Fertilizers

Fertilizers are optimally classified into different categories based on their nature, source, and physical state. Upon application in the soil, fertilizers have the capacity to alter the soil pH. Therefore, based on their nature, they can be classified as acidic or basic fertilizers (Randive et al. 2021). Acidic residue is deposited in the soil due to acidic fertilizers and alkaline residue is deposited in the soil due to basic fertilizers. Fertilizers are further classified as natural, chemical, and microbial fertilizers. Natural biofertilizers are also known as traditional biofertilizers which are obtained from natural biogenic materials like cow dung or urine (Raj et al. 2014). These include fish-based biofertilizers, cold salt fertilizers, plant waste matter, composting matter, etc. which are rich in macronutrients such as potassium, nitrogen, and phosphorous (Randive et al. 2021). Chemical biofertilizers, on the other hand, are known as inorganic or synthetic fertilizers manufactured in the factories. Chemical synthesis is used for the production of these. Mixed fertilizers are made up of a number of components, either used alone or in conjunction with other ingredients. However, in reality, fertilizers rich in phosphate, potassium, and nitrogen are frequently utilized (Nangle et al. 2017). The most appropriate approach is biofertilizers which are also known as microbial inoculants or microbial fertilizers. These biofertilizers are made of microorganisms, which create an environment in the soil that is favorable to plant life and help in the synthesis of plant nutrients. These include nitrogen fixing biofertilizers such as *Rhizobium*, *Azolla*, *Azospirillum*; phosphorous solubilizers like

Bacillus, *Pseudomonas*; zinc solubilizers like *Thiobacillus*, *Thioxidans*, and organic matter decomposers like *Trichoderma* (Nosheen et al. 2021).

Biofertilizers based on the physical state includes carrier-based biofertilizers and liquid biofertilizers. In the production of solid carrier-based biofertilizers, a carrier is used as a medium to carry the required microorganism while maintaining their viability during the transportation. Peat, charcoal, and perlite are some of the commonly used carriers (Amenaghawon et al. 2021). A variety of carrier materials including sodium alginate, compost, peat, clay, wheat bran, wood ash, rice husk were tested. Sodium alginate was the most effective among all (Sakpirom et al. 2021). A suitable carrier must have high water holding capacity, be easy to sterilize and transport, have low production cost and should not be toxic to microorganisms. In addition to these, carriers must also possess excellent adhesion quality for seed inoculation (Deaker et al. 2004). An appropriate carrier material must be used to ensure adequate compatibility and quality. A study conducted to determine the best carrier material to support the microbial population of dry land inoculants (*Bacillus* sp., *Delftia tsuruhatensis* strain D9, and *Delftia sp*. strain MS2As2) was carried out. It was reported that the most efficient carrier material for preserving the quantity of live inoculant cells was a mixture of 3% molasses, 1% potassium sorbate, 3% glycerol along with 1% Tween-20 enhanced with 1% nutrient. This formulation met the primary requirements for biofertilizers and was able to maintain the inoculant population up to the 12th week, with a viable count of 21.60×10^8 CFU mL^{-1} (Harahap et al. 2023). Liquid biofertilizers are liquid bioformulations that have the required microorganism present in a dormant state. Liquid biofertilizers are known to be better than carrier-based biofertilizers because of longer shelf life and better tolerance to unfavorable conditions (Chaudhary et al. 2020). Moreover, the chances of contamination in the liquid biofertilizers are lower than that of carrier-based biofertilizers. The packaging material cost required under carried based is also saved here deeming it cost effective. Under the favorable conditions of soil, these dormant formulations become active and readily multiply in the rhizospheric zone of the plants. Seed treatment, root dipping, and direct soil application are the ways a liquid biofertilizer is used (Singh et al. 2021). The results of a study for the evaluation of shelf life showed that, in comparison to liquid-based biofertilizers, carried-based biofertilizers had higher contamination and low viable count. Liquid biofertilizer was able to maintain a sufficient viable count for up to 6 months, whereas carrier-based biofertilizers were able to maintain the viable count for only up to 3 months (Shravani 2019).

1.3 Advances in Biofertilizers

Biofertilizers include a range of microorganisms that colonizes the rhizospheric zone easing the uptake of nutrients and making them more accessible to the root hairs of the plants. Biofertilizers are widely recognized for their reliability and affordability. They are the most effective tools for agricultural production and a safe substitute for the toxic artificial fertilizers (Dasgupta et al. 2021). The solution for a sustainable agriculture demands for an advancement in the present day biofertilizer practices to enhance the biofertilizer technology.

1.3.1 Nanofertilizers

Nanofertilizers involve the use of nanoparticle technology or nanomaterials. They administer the required nutrients to a plant at a nano-scale. They enhance productivity by promoting plant growth and development (Chhipa 2017). These nanoparticles are less than 100 nm in size to serve the purpose of supplying vital nutrients. They address the issue of relatively low nutrient use efficiency (NUE) and hazardous environmental impact due to synthetic fertilizers which is a major hindrance in practicing sustainable agriculture (Dimkpa and Bindraban 2017). Appropriate application of nanofertilizers provides nutrients to the plant in a regulated manner while also reducing environmental impact, increasing NUE, minimizing leaching and volatilization. These fertilizers which are also known as "smart fertilizers" are now being recognized as a potential alternative against the conventional ones because their nanomaterials are highly reactive upon interaction with fertilizers enhancing their absorption and uptake of essential nutrients which are required for effective plant growth (Prasad et al. 2017). Additionally, when combined with microorganisms as "nanobiofertilizers," these nanofertilizers can be used to increase abiotic stress tolerance and offer the host with additional advantages (Zulfiqar et al. 2019). Combining nanofertilizers and biofertilizers has several benefits and creates new possibilities for sustainable agriculture. In addition to enhancing agricultural production, nanobiofertilizers are also known to reduce mineral loss and maximize yield. Moreover, by employing microbial integration through a bio-organic component, enhanced plant growth, rhizoremediation, and disease resistance is observed. Nanobiofertilizers also improve soil fertility and moisture levels along with providing the plants with required essential nutrients (Fazelian and Yousefzadi 2022). In a study, a nanobiofertilizer was developed using biosynthesis of onion silver nanoparticles (AgNP'S) using the onion extract as a reducing agent. When compared to control, plants fed with 15 ml/l of nanobiofertilizer had greater fresh weight and vigor. Tomato and brinjal plants benefit greatly from the application of the synthetic nanobiofertilizer made from onion extract. The overuse of chemical fertilizers, contamination of the environment and expenditures associated with farm management can all be decreased by using these kinds of nanobiofertilizers (Gosavi et al. 2020). A lightweight nanobiofertilizer was developed using immobilized bacterial endospores (*Panebacilluspolymyxa*) in activated carbon beads and Fe-carbon nanofibers (Fe-CNFs) coated with acylated homoserine lactone (AHL). It was tested on both leguminous (*Cicer arietinum*) and non-leguminous (*Triticum aestivum*) plants. The findings demonstrate a significant ($p < 0.05$) increase in biomass, root length, chlorophyll, and protein contents in plants grown for 30 days following nanocomposite biofertilizer (NCB) supplementation. Furthermore, after 21 days of growth with NCB, the plants showed a considerable ($p < 0.05$) ability to withstand root rot in wheat and chickpea caused by *Fusarium oxysporum* sp. *Cicero* and *Cochliobolus sativus*, respectively (Gahoi et al. 2021).

Nanofertilizers are made using the material obtained from synthetic fertilizers or extraction from plant reproductive or vegetative parts by means of chemical, mechanical, and physical processes (Qureshi et al. 2018). The physical process such as radiation, ultra-sonication, mechanical pressure, and thermal or electrical energy are used for melting and condensing for fabrication of nanoparticles, whereas the chemical process includes sol-gel method, micro-emulsion technique, hydrothermal synthesis polymer synthesis, plasma enhanced deposition method, and chemical vapor synthesis (Satyanarayana and Reddy 2018). The nanoparticles are synthesized via top-down approach (where bulk particles are broken into smaller bits using external force) or bottom-up approach (where gas and liquid molecules are combined and gathered together). Other than this, polymers are used for stabilizing and encapsulating these nanofertilizers. Nutrients are encapsulated by covering them in a thin layer of nanoparticle (Yaseen et al. 2020). Chemically synthesized nanoparticles were characterized and administered using randomized complete block design (RCBD) and were administered in plants using soil and foliar application. The results showed that ZnO as a nanofertilizer improved maize cultivar growth and extract production and has the potential to be used as a nanofertilizer for crops grown in Zn-deficient soils to increase crop quantitative and qualitative value (Azam et al. 2022).

Another approach for the production of nanofertilizers is the biosynthesis approach where biological process is used for the fabrication of nanofertilizers. The biological method is much more environment friendly dependable. Molecules such as pigments, proteins, enzymes, phenolic, and alkaloid compounds obtained from microbes and plants are used for nanoparticles production (Prasad et al. 2017). Larger surface area, increased reactivity, and improved contact are some of the advantages provided via this technique. Stabilizing agents such as proteins, carbohydrates, enzymes, and phytochemicals such as flavonoids, terpenoids, phenolics, and cofactors are also used. Additionally, it was found that a variety of microorganisms and plant extracts might be used to accomplish the biosynthesis of a nanofertilizer (Yaseen et al. 2020). An increasing amount of focus is being paid to pursue for newer techniques for the biosynthesis of nanofertilizers.

Nanotechnology has enormous potential to change many facets of human life but continuous improvement, ethical measures and safety must be applied before fabricating a nanoparticle. Risks such as nanoparticle toxicity associated with the implementation of this technology must be assessed before the application. As a result, a comprehensive research and series of experiments must be conducted before fully accepting this technology (Fatima et al. 2021).

1.3.2 Biofilm-Based Biofertilizers

Biofertilizers are biological products which can potentially reduce dependency on chemical fertilizers. However, the major drawback is the inadequate survival of viable cells in the soil. A feasible cue would be using the cells which are immobilized in biofilms as this would ensure protracted cell survival and better suitable environment for the bacteria and its metabolites to interact with the plant. Biofilm-based biofertilizers are another potential alternative to synthetic or conventional fertilizers. Biofilm is a structure developed by microbial assemblage encased in a matrix that the bacteria build on their own for the ease of communication. In terms of applications, they are already in use for purification of water by entrapment of pathogens in the polluted water and processes like bioremediation (Armbruster and Parsek 2018). Biofilm application is done to encourage plant growth and its development.

In terms of soil, fungus and bacteria live together and share micro-niches. The metabolic processes of an microorganism is influenced by other microorganisms. This co-existence positively impacts the plant growth and associated ecosystem. Comparing mono or mixed cultures without biofilm development to fungal–bacterial biofilms, which are bacterial biofilms connected to fungal surfaces, has demonstrated improved nutrient uptake, plant growth, and tolerance against environmental stress (Hassani et al. 2018). In the rhizospheric zone, biofilms aid in enhancing water retention, bacterial biomass, stimulating the exudation of roots for their response against stress (Kasim et al. 2016). Biofilms biodegrade heavy metals and organic contaminants in soil. Their application involves either direct spraying on the aerial regions of the plants as mixed inoculum or as beneficial bacterial biofilm inoculum. They can also be incorporated into the soil as inocula (Gui et al. 2017). As a result, these interactions not only promote plant development but also enhance soil health by increasing nutrient cycling. Multiple-specie biofilms need more attention because they have the potential to create polysaccharides and other bioactive substances that have a favorable influence on soil health and plant growth on a bigger scale (Pandit et al. 2020).

There is not enough naturally occurring biofilms in the soil to make a noticeable difference. Therefore, it is essential to generate biofilms in vitro and then use them as biofertilizers or biofilm-based fertilizer (BFBF) to increase agricultural output in an environmentally responsible way. In a study conducted with regard to rice production, application of biofilm-based fertilizer (BFBF) reduced the requirement of chemical fertilization to up to 50% while simultaneously boosting grain yield by up to 25%. These results contributed toward a healthy relation of BFBF's with microorganisms, plants, and soil (Premarathna et al. 2021).

Using glycerol as the only carbon source, the planktonic *Bacillus subtilis subsp. spizizenii* was able to create a thick biofilm in a study conducted on *Lactuca sativa* biofertilization. The immobilized cells portrayed positive PGPR results, increasing the root growth by 59% and aerial growth by 39% (Galelli et al. 2015).

Another aspect to biofilm-based biofertilizers is sequestration of organic carbon. Research is in progress for analyzing the increase in soil organic carbon while also enhancing the crop yield by using these biofilm biofertilizers. In four districts of Sri Lanka, the use of biofilm biofertilizer was compared with prevalent practice of applying chemical fertilization. Infrared spectroscopy was implied to study the stabilization of sequestered soil carbon. The outcome was that biofilm biofertilizer produced soils with stronger organomineral complexation. Soil respiration was also reduced down to about 40% in comparison to standard chemical fertilization. This could be because of enhanced mineral surface-reactive metabolites and low priming effect, respectively, in biofilm biofertilizer which contributed in mitigating the effects of climate change. In summary, farmers can effectively replace their use of chemical fertilizers with the biofilm biofertilizer practice upon adequate research (Premarathna et al. 2023).

1.3.3 Encapsulation Technology

To be employed as slow release fertilizers (SRF's), encapsulation is done in a compound which is made from natural or synthetic polymer. The newest technology in fertilizer development is polymers-coated nutrient fertilizers. Superabsorbent polymers have garnered significant since they utilize less water decreasing the load of frequent irrigation. These polymers are also effective in lowering the rate of plant mortality. Additionally, they increase plant growth rates and soil fertilizer retention (Ma and Wen 2020). This technology created using synthetic polymers portrays impressive outcomes in terms of continuous yet slow release but this ongoing utility is limited by their high cost and inability to decompose. To address these issues, biodegradable polymers are utilized as an environmentally friendly substitute for synthetic polymer-based ones. These polymers are derived from naturally occurring substances that are safe for living things to consume, such as corncob and cotton stalks (Iftime et al. 2019).

Polymer seed coating is another popular approach where a seed is coated in a polymer to protect it from biotic and abiotic stress conditions. Crop productivity is significantly influenced by the quality of the seeds. To improve seed quality, techniques such as treating seeds, improving the soil, modifying genes, etc. must be applied. In order to produce healthy crops, seed treatment serves as the first line of defense against soil-borne and seed-borne infections. The term "seed treatment" refers to the act of applying or covering seeds with biological or chemical agents. It is a seed coating in which the polymer, which contains various materials (pesticides, nutrients, etc.), sticks firmly to the seed resulting in the increase in nutrient use efficiency and reduced pollution (Krishnamoorthy and Rajiv 2018). Biopolymer like chitosan is extensively used in this method. In another example a mixture made of polyethylene glycol and chitosan that was used as a seed coating in the carrier for *Trichoderma* strains (Abd El-Aziz et al. 2022).

1.4 Constraints in Biofertilizer Technology

Despite being an organic source of almost all important crop nutrients, the current application rate of biofertilizers is still limited. Lack of information, insufficient extension services, global, national, regional, and local agricultural policies, institutional changes, etc., are key barriers to the implementation of sustainable agricultural methods. Therefore, it becomes important to discuss the constraints faced by various farmers for the use of biofertilizers and comprehend the reasons behind the same (Linares Quero et al. 2022).

One of the major limitations stems from the lack of knowledge and understanding among farmers about the use and importance of biofertilizers. Lack of knowledge regarding the concentration, duration, and manner of application leads to limited use of biofertilizers in modern agricultural fields. Other limitations arise from lack of suitable organic farming training to farmers and insufficient organic farming expertise making it a significant barrier to the implementation of organic farming (Jangid et al. 2012). Recent studies have concluded that majority of small-scale farmers have primary to graduate education, medium land holding, low scientific orientation, and lower mass media exposure relating to the use of biofertilizers. Studies concluded that a large number of small-scale farmers indicated a lack of technical expertise in using biofertilizers and thus it can be stated that biofertilizer consumption may be increased to great extent if they were trained about the proper practices surrounding its use (Diptesh and Chauhan 2016).

According to various studies, lack of awareness as well as familiarity about the efficacy and effectiveness of biofertilizers compared to the tried and tested inorganic fertilizers leads to further inability of farmers to incorporate biofertilizers to their crops. Limited capacity of government's ambiguous market support also leads to a reduced awareness of organic biofertilizer making them unable to compete with the well-established inorganic fertilizer industry and the massive marketing strategies applied by private fertilizer companies (Baconguis et al. 2012).

Institutional restrictions and a shortage of competent human capital in research institutes and private enterprises have always been key impediments to mass production and commercialization of many biotechnological advances. With the lack of technical advances, poor grade inoculants are produced without a fundamental grasp of microbiological processes also leading to shorter shelf lives. Other constraints in technology such as a scarcity of high-quality carrier material as well as a lack of trained and technical staff in production facilities affect the overall production and application of biofertilizers (Dhar et al. 2009).

To successfully commercialize biofertilizers, there is a need to broaden the existing resource base by locating and incorporating more viable strains, work on enhancing shelf life of the product, develop better production technologies and better-quality control systems (Rai et al. 2023). Many biofertilizers are required to be developed and constantly modified according to the environmental conditions. Variations in seasonal demands, simultaneous cropping activities, and differing time periods of sowing or planting in different locations lead to major environmental constraints on the use of biofertilizers (Kumar et al. 2017). Due to these considerable changes in agroclimatic conditions and different requirements for specific soils (alkaline, sodic, acidic, etc.) varying throughout the country, single biofertilizer cannot be produced to give equal productivity everywhere.

To address the obstacles, there is a need to improve dissemination of information, strengthening the government's role in fostering collaboration among various components of the bio-innovation system. Farmers currently have a significant need for information and they are eager to learning more about the practice of utilizing biofertilizers and how they relate to controlling plant pests and diseases, improving crop quality and fostering competitiveness. The benefits of employing biofertilizers should be aligned with the information that farmers require the most from pre-to post-harvest (Kassem et al. 2021). Participation of agricultural cooperatives to provide subsidies on organic fertilizers and enhance the existing awareness can have favorable effects in influencing farmers toward choosing biofertilizers over chemical fertilizers (Wang et al. 2018).

1.5 Conclusions and Future Prospects

The estimated value of the global biofertilizer market is 2.3 billion US dollars, with projections showing a 3.9 billion-dollar growth by 2025. Biofertilizer technology despite being having potential still faces significant obstacles preventing their widespread usage in agriculture (Mitter et al. 2021). Efforts must be given to investigate the success and failures in biofertilizer technologies. For this there is a need of high levels of innovation and active participation in cutting-edge scientific research and development, public demonstration programs, and encouraging private organizations and new policymakers to take an active role in the production and marketing of biofertilizer (Kumawat et al. 2021) Working on the limitations will pave the way for advanced biofertilizer technology giving the agriculture more sustainable approach.

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Futuristic Approaches in Biofertilizer Industry: Challenges, Opportunities, and Future Directions

2

Hardeep Kaur, Shinar Athwal, and Kashish Garg

Abstract

Over the years conventional fertilizers have been replaced by biofertilizers owing to their negative impact on humans and the environment. Biofertilizers are an integral part of agricultural industries as they are responsible for maintaining the soil ecosystem and increasing the crop yield. Also, bio-based fertilizers have environment-friendly aspects that promote sustainable agriculture. The biofertilizers industry is at its boom and is experiencing an addition of new approaches with time. The future of organic fertilizers lies in the growth of microbial, nano-sized, and liquid biofertilizers as they have the potential to bring a green change in the crop industry. The most used microbial biofertilizers are nitrogenous (rhizobacteria, Azospirillum), fungal (mycorrhizae, penicillium), and endophytic (Pseudomonas, Rahnella). Nanobiofertilizers are composed of nanoparticles (gold, silver, iron-oxide, zinc) or nanocomposites (nanoforms of organic compounds). Concerning liquid biofertilizers the commonly used solutions (solubilizers) are nitrogen, potassium, and phosphate, which are the essential components of the fertilizers. This review gives a deep insight into the emerging forms of biofertilizers (microbial, nano, and liquid) along with their impact on the soil system and crop production. All the mentioned forms exhibit properties that increase and/or maintain the microbial, and nutrient balance in the soil consequently improving the crop yield. Not only this but also these biofertilizers help to preserve the essential components of nature and thus promote green agricul-

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ture. Although the upcoming biofertilizers have certain limitations which will be discussed in the articles along with this the futuristic direction will also be reflected upon to deal with the challenges faced while employing biofertilizers.

Keywords
Biofertilizers · Nano fertilizers · Microbial fertilizers · Liquids fertilizers · Sustainable agriculture · Green approach

2.1 Introduction

Biofertilizers play a pivotal role in the progress of the sustainable agriculture approach. It is a technique that uses fertilizers but in the form of biological matters to increase the overall positive impact it has on the environment and humans (Mahanty et al. 2017; Malusà et al. 2016). Chemical fertilizers are hazardous and responsible for causing natural damage and negatively impacting human health so biofertilizers present an effective way to deal with this problem. Fertilizers are important sources of microbial communities and plant growth so it is important to achieve better development of crops (Mącik et al. 2020; Mahapatra et al. 2022). Over the years various research studies have been performed to understand the applicability of biofertilizers and raw materials which can be used to develop green fertilizers (Nosheen et al. 2021). The fertilizers industry is the most important one in the agricultural sector as it can promote the shoot length, root length, leaf size, quality, and yield of the crop (Kaur and Purewal 2019; Reddy et al. 2020; Pirttilä et al. 2021; Mahmud et al. 2021; Singh et al. 2021) (Fig. 2.1). Since biofertilizers have so many advantageous points so considerable amount of research has been conducted on the development of efficient biofertilizers (Anli et al. 2020; Dal Cortivo et al. 2020; Du et al. 2022).

Innovations in the field of biofertilizers are related to the advancement in the material properties of the fertilizer. Improvised fertilizers used in today's scenario are based on microbes, nanotechnology, and liquid sprays (Seenivasagan and Babalola 2021; Puglia et al. 2021). Microbial fertilizers such as nitrogen-fixing, fungus, and endophytic organisms are used in biofertilizers to fix atmospheric nitrogen, solubilize phosphate, and increase the availability of minerals in the soil environment (Riaz et al. 2021; Dasgupta et al. 2021; Zainuddin et al. 2022; Negi et al. 2021). Additionally, the integration of nanotechnology into fertilization materials brings about effective results. Gold, zinc, iron, manganese nanoparticles, and other nanocomposites can be utilized to improve the overall development of the host (Bhattacharyya et al. 2020; Dong et al. 2019; Sambangi et al. 2022). Liquid fertilizers have also gained considerable attention as they are handy and easy to use and commercially available as potassium, phosphorus, and nitrogen solubilizers (Ma et al. 2020; Barzee et al. 2019; Sharma et al. 2023a). This article includes the recent advancements in biofertilizers, namely, microbial, nano, and liquid along with this the limitations of the same have been discussed in detail. Biofertilizer is a way of promoting green agriculture to follow the path of sustainable development.

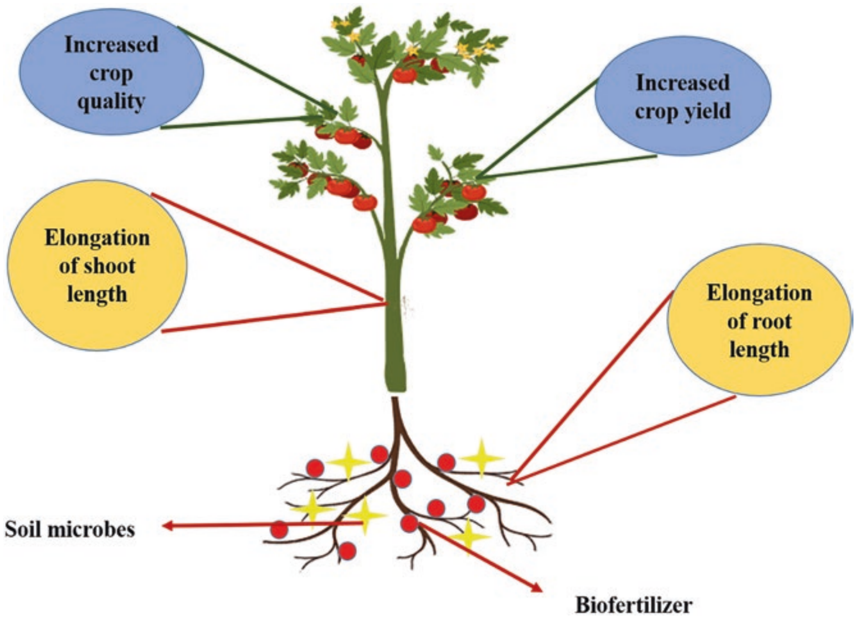

Fig. 2.1 Biofertilizers can promote the shoot length, root length, leaf size, quality, and yield of the crop

2.2 An Emerging Trend of Microbes in the Biofertilizer Industry

The microbes that are widely used to form biofertilizers are nitrogen-fixing, fungus, and endophytic organisms. Nitrogen-fixing bacteria includes *Azotobacter, rhizobium, Azospirillum, and Klebsiella*, fungal species consists of arbuscular mycorrhiza fungi, *Trichoderma*, and saprophytic species while endophytic species includes *Bacillus* sp. and *Piriformospora indica* as shown in (Fig. 2.2).

2.2.1 Types of Microbial Fertilizers

2.2.1.1 Nitrogen-Fixing

Different microbial species can be used to develop enhanced biofertilizers such as *Azotobacter, rhizobium, Azospirillum, and Klebsiella*. These fertilizers help to fix atmospheric nitrogen with the associated plants and are capable of uptake fixed nitrogen along with decreased losses by leaching, volatilization, and denitrification. Several nitrogen-fixing bacteria have been used to develop biofertilizers (Thomas and Singh 2019). *Azotobacter* species uses classic molybdenum-containing enzymes for biological nitrogen fixation (BNF). It also can produce more than one alternative nitrogenase in the absence of molybdenum. Some studies depicted that *Azotobacter* increases nitrogen accumulation as it produces phytohormone-like compounds that