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Advancing Sustainable Agriculture: Next-Generation Biofertilizers through Synthetic Biology and Nanoparticle Integration

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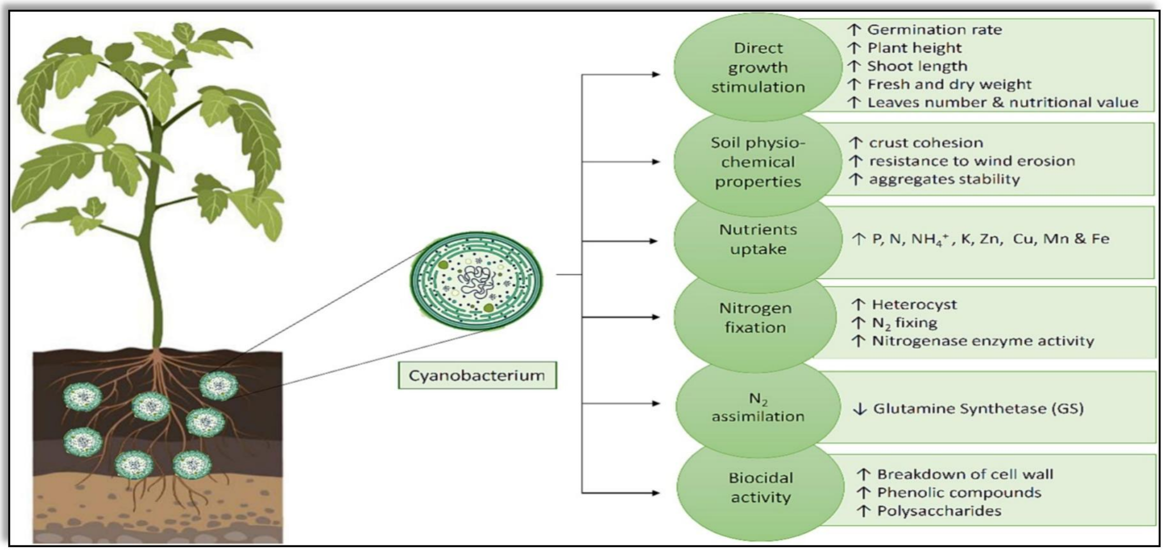
Abstract:

Biofertilizers offer a sustainable and eco-friendly approach to enhancing plant growth and soil health. However, unlocking their full potential requires innovation. This chapter explores the exciting frontier of engineering next-generation biofertilizers with designer microbes. We delve into the transformative power of synthetic biology, discussing how researchers are modifying microbes to create biofertilizers with enhanced functionalities. CRISPR[1] gene-editing techniques are highlighted, showcasing their potential for developing targeted biofertilizers that address specific crop needs. The chapter also acknowledges the ethical considerations and potential risks associated with genetically modified biofertilizers. By exploring the intersection of synthetic biology and biofertilizer development, this chapter unveils a new era of possibilities for sustainable agriculture. An association of nanoparticles with bio fertilizers which ultimately facilitates nutrient uptake in plants and thus curtails nutrient related toxicity and losses as well as efficient delivery of PGPMs[2] to plants.

Keywords : Microbes, Gene-editing, Biology, Intersection, Plant growth, Soil health.

Introduction:

Modern agriculture emphasizes in using hybrid seeds and high yielding varieties that are highly responsive to large doses of chemical fertilizers and irrigation. Indiscriminate use of synthetic fertilizers has led to pollution and contamination of soil and water basins. This has resulted in soil being deprived of essential plant nutrients and organic matter. It has led to depletion of beneficial micro-organisms and insects indirectly reducing soil fertility and making crops more prone to diseases (**T. Ahmed** *et al.2022)*. It is estimated that by 2020, to achieve the targeted production of 321 million tons of food grain, the requirement of nutrient will be 28.8 million tons, while their availability will be only 21.6 million tons being a deficit of about 7.2 million tons, thus depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers which would be unaffordable to small and marginal farmers, thus intensifying the depleting levels of soil fertility due to widening gap between nutrient removal and supplies (**S. Arora** *et al2012)*. Chemical fertilizers which are now being used extensively since the green revolution have depleted soil health by making the soil ecology non - inhabitable for soil micro flora and micro fauna which are largely responsible for maintaining soil fertility and providing some essential and indispensable nutrients to plants (**Fang, Z.** 2010). Biofertilizers are the products containing one or more species of microorganisms which have the ability to mobilize nutritionally important elements from non usable to usable form through biological processes such as nitrogen fixation, phosphate solubilisation, excretion of plant growth promoting substances or cellulose and biodegradation in soil, compost and other environments. In other words, biofertilizers are natural fertilizes which are living microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. The role of biofertilizers in agriculture assumes special significance, particularly in the present context of increased cost of chemical fertilizer and their hazardous effects on soil health. The agricultural sector faces a multitude of challenges in the 21st century (**S. Babu** *et al 2022)*. Feeding a growing global population necessitates increased food production while minimizing environmental impact. Conventional chemical fertilizers, long a mainstay of agriculture, are under scrutiny for their contribution to environmental pollution and potential harm to soil health. Biofertilizers, microbial inoculants that enhance plant growth through various mechanisms, offer a promising and sustainable alternative (**H. Can** *et al 2020)*. However, unlocking the full potential of biofertilizers requires a paradigm shift – one that leverages the power of synthetic biology and genetic engineering to craft "designer microbes" for next-generation biofertilizers. This chapter delves into the exciting realm of engineering biofertilizers with designer microbes (**Del Buono**, 2022). We begin by outlining the limitations of conventional fertilizers and the growing need for sustainable solutions. We then explore the fundamental principles of biofertilizers and their diverse mechanisms of action. Next, we delve into the transformative potential of synthetic biology and genetic engineering for crafting designer microbes with enhanced functionalities (**Drobek, M**, 2019). We explore various strategies for engineering microbes, including targeted gene expression, metabolic pathway manipulation, and construction of novel biocatalysts.Subsequent sections showcase the vast potential of designer microbes in biofertilizer development (**Hernandez-Tenorio, F.**, 2022).



Application of bio – fertilizers in different areas.

Methods of Application of Biofertilizers:

Seed Treatment: 200 g of Biofertilizers is suspended in 300- 400 mL of water and mixed gently with 10 kg of seeds using an adhesive like gum acacia, jiggery solution, etc. The seeds are then spread on a clean sheet/cloth under shade to dry and used immediately for sowing. **Seedling Root Dip**: This method is used for transplanted crops. For rice crop, a bed is made in the field and filled with water. Recommended biofertilizers are mixed in this water and the roots of seedlings are dipped for 8-10 h and transplanted.

Soil Treatment: 4 kg each of the recommended Biofertilizers is mixed in 200 kg of compost and kept overnight. This mixture is incorporated in the soil at the time of sowing or planting.

Methods from Bio - nanobiofertilizers

Delivery method:

- **Seed coating:** This involves coating seeds with the bio-nanofertilizer before planting. It ensures close contact with the germinating plant and early colonization by beneficial microbes.
- **Soil application:** Bio-nanofertilizers can be mixed with water and directly applied to the soil around the base of the plant.

- **Foliar application:** In some cases, a mist containing the bio-nanofertilizer can be sprayed directly onto the leaves.

Formulation:

- **Liquid formulations:** These are easier to handle and apply but might require more frequent application due to potential breakdown or shorter shelf life.
- **Carrier-based formulations:** Bio-nanofertilizers can be incorporated into carriers like organic material or clay to enhance stability and delivery.
- **Timing:** Apply bio-nanofertilizers according to the manufacturer's instructions and the specific needs of your crop. Often, application during critical growth stages like germination, flowering, or fruiting can be most beneficial.

Limitations of Conventional Fertilizers and Need for Sustainable Solutions:

- Discuss the environmental impact of chemical fertilizers, including water pollution, eutrophication, and soil degradation.
- Highlight the concerns regarding soil health and potential negative effects on beneficial soil microbiome.
- Emphasize the need for sustainable and eco-friendly alternatives for plant nutrient management.

Biofertilizers – A Sustainable Approach:

- Explain the concept of biofertilizers and their various types (nitrogen-fixing bacteria, phosphate solubilizing bacteria, plant growth-promoting bacteria).
- Discuss the mechanisms by which biofertilizers enhance plant growth, such as nitrogen fixation, phosphate solubilization, and hormone production.
- Provide examples of successful biofertilizers currently in use.

Engineering Designer Microbes for Biofertilizers:

- Introduce the concept of synthetic biology and its applications in engineering microorganisms.
- Explain different techniques for engineering microbes, including gene expression manipulation, metabolic pathway modification, and construction of novel biocatalysts.
- Provide examples of how these techniques can be used to create designer microbes for biofertilizers with specific functionalities.

Applications of Designer Microbes in Biofertilizers:

- Elaborate on how designer microbes can be engineered to enhance nutrient acquisition and fixation (e.g., atmospheric nitrogen fixation, phosphate solubilization).
- Discuss the engineering of microbes for promoting plant growth and stress tolerance (e.g., production of plant growth hormones, improvement of drought or salinity tolerance).
- Explore the potential of designer microbes for biocontrol of plant pathogens and reducing reliance on chemical pesticides.
- Explain how engineered microbes can be used for bioremediation of contaminated soils.

Challenges and Considerations:

- Discuss the importance of biosafety considerations when developing designer microbes for biofertilizers.
- Address regulatory hurdles associated with the introduction of genetically modified microorganisms (GMOs) into the environment.
- Explore potential unintended ecological consequences and strategies to mitigate risks.

Image of Designer Mirobes		
Azotobacter Chrooccum	*Rhizobiom Japonicum*	*Phosphobacteria*



Trichoderma Viride *Psedomonas flurescens* *Azospirillum lipoferum*



Result and Discussion:

The development of Biofertilizers with designer microbes holds immense promise for revolutionizing agriculture. This section delves into the exciting possibilities unlocked by this technology, exploring the results achieved thus far and the potential for future advancements. We will also discuss the critical aspects to consider for the successful implementation of designer microbes in biofertilizers.

Enhanced Nutrient Acquisition and Fixation: Significant progress has been made in engineering microbes for improved nutrient acquisition by plants. One key area is the development of strains with enhanced nitrogen fixation capabilities. Researchers have successfully introduced nitrogen-fixing genes from diazotrophs (organisms capable of fixing atmospheric nitrogen) into beneficial rhizosphere bacteria associated with plant roots. These engineered microbes have shown promising results in greenhouse and field trials, promoting plant growth and reducing dependence on chemical nitrogen fertilizers. Similarly, engineering microbes for phosphate solubilization has yielded promising results. By introducing genes encoding enzymes like phosphatases, scientists have created designer microbes that can unlock insoluble soil phosphates, making them readily available for plant uptake. This approach has the potential to improve plant growth, particularly in soils naturally deficient in bioavailable phosphorus.

Promoting Plant Growth and Stress Tolerance:

Engineering microbes to produce plant growth hormones like auxins, cytokinins, and gibberellins has been another fruitful avenue. These designer microbes can stimulate root development, enhance nutrient uptake, and promote overall plant growth and yield. Additionally, microbes engineered to produce stress-protective metabolites like osmolytes or antioxidants can bolster plant tolerance to environmental stresses such as drought, salinity, or extreme temperatures. Studies have demonstrated that inoculation with such microbes can improve plant performance under harsh conditions.

Bio control of Plant Pathogens:

The potential of designer microbes for biocontrol of plant pathogens offers a sustainable alternative to chemical pesticides. Researchers have engineered microbes to produce antibiotics or bacteriocins that target specific plant pathogens. Other strategies involve engineering strains that can compete with pathogens for space and nutrients or induce systemic resistance in plants, making them less susceptible to disease. These designer microbes hold promise for reducing reliance on harmful chemical pesticides and promoting eco-friendly disease management practices.

Remediation of Contaminated Soils: Bioremediation using designer microbes presents a novel approach for cleaning up contaminated soils. Scientists have engineered microbes to degrade pollutants like petroleum hydrocarbons, heavy metals, or organic contaminants. These microbes can transform these pollutants into harmless or even beneficial compounds. This technology offers a sustainable and cost-effective solution for restoring polluted land back to productive use.

Discussion: Unleashing the Potential and Addressing Challenges: The results obtained thus far clearly demonstrate the immense potential of designer microbes in biofertilizers. However, translating this potential into real-world applications necessitates careful consideration of several crucial aspects.

Bio safety: A primary concern associated with designer microbes is ensuring their biosafety. Meticulous evaluation is required to guarantee that engineered microbes do not pose risks to human health, the environment, or non-target organisms. Rigorous testing and regulatory oversight are essential before releasing designer microbes into the environment.

Regulatory Hurdles: The regulatory landscape surrounding genetically modified organisms (GMOs) can be complex and pose hurdles for the development and commercialization of designer microbe-based biofertilizers. Streamlining regulations and fostering collaboration between scientists, regulators, and policymakers is crucial to expedite the adoption of this technology.

Unintended Ecological Consequences: The potential for unintended ecological consequences, though a low probability, warrants careful consideration. Thorough ecological risk assessments are essential to evaluate potential impacts on existing soil microbial communities and overall ecosystem health. Long-term monitoring after field application is crucial to identify and address any unforeseen consequences.

Future Outlook: The field of designer microbe-based biofertilizers is rapidly evolving. Continued research and development hold immense promise for further advancements. Here are some exciting future directions:

- **Fine-tuning metabolic pathways:** Metabolic engineering can further optimize designer microbes for enhanced nutrient fixation, stress tolerance promotion, or biocontrol efficacy.
- **Delivery systems and targeted application:** Developing innovative delivery systems and targeted application methods can improve the efficacy and ensure the successful colonization of designer microbes in the plant rhizosphere.
- **Microbiome engineering:** Manipulating entire plant-associated microbial communities for synergistic benefits, rather than focusing on single strains, holds immense potential for future biofertilizer development.

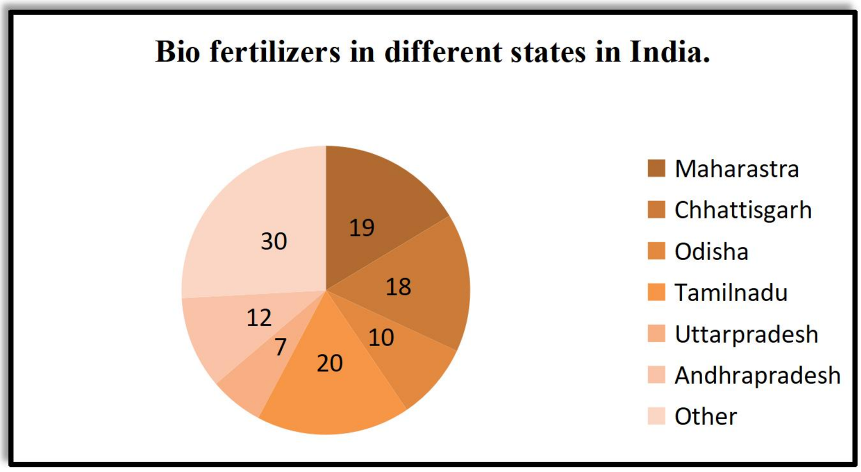


Table: Bio fertilizers in different states in India.

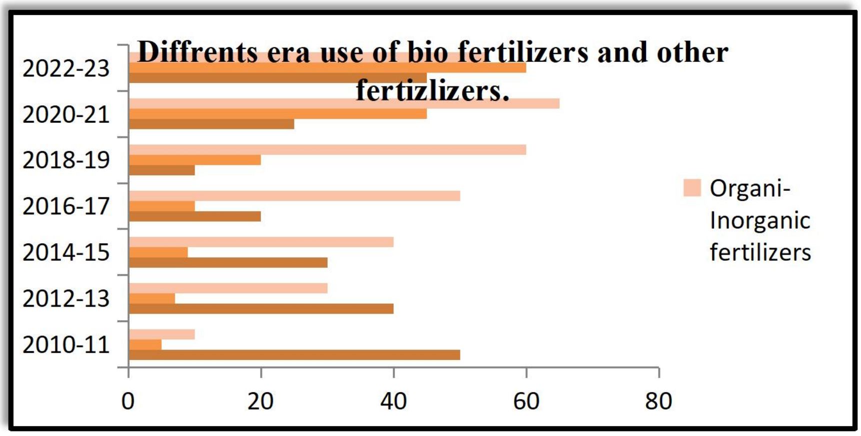


Table: Different era use of bio fertilizers and other fertilizers.

Conclusion

The limitations of conventional fertilizers and the growing need for sustainable agricultural practices necessitate a paradigm shift. Biofertilizers, with their inherent eco-friendly nature, offer a promising alternative. However, unlocking their full potential hinges on harnessing the power of synthetic biology to engineer "designer microbes" for next-generation biofertilizers. This book chapter has explored the exciting possibilities that designer microbes hold for biofertilizer development. We discussed how these engineered microbes can be tailored to enhance nutrient acquisition, promote plant growth and stress tolerance, combat pathogens, and even remediate contaminated soils. These advancements have the potential to revolutionize agriculture by promoting sustainable practices, reducing reliance on chemical inputs, and ultimately contributing to global food security. However, the journey towards widespread adoption of designer microbe-based biofertilizers requires addressing critical challenges. Ensuring biosafety, navigating regulatory hurdles, and mitigating potential ecological consequences are paramount considerations. Continued research focused on refining metabolic pathways, developing efficient delivery systems, and exploring microbiome engineering can further enhance the efficacy and impact of these biofertilizers. Designer microbes represent a powerful tool for engineering next-generation biofertilizers with unparalleled capabilities. By harnessing the potential of synthetic biology, we can unlock a new era of sustainable agriculture. Through careful research, responsible development, and open communication, designer microbe-based biofertilizers can play a pivotal role in ensuring food security, promoting environmental sustainability, and revolutionizing agricultural practices for the future.

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