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REVIEW ARTICLE OPEN ACCESS

Microbial Engineering for a Greener Ecosystem and Agriculture: Recent Advances and Challenges

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Abstract

Tremendous increase in anthropogenic activities and natural disasters have created long term negative impacts to the crop productivity as well as on our ecosystem. In the debate regarding the ongoing ecosystem fluctuations, there is a need to explore an efficient, cost-effective, target-oriented and less manpower based technologies for sustainable development. Microbial engineering provides a better solution for the growth of a healthy environment and higher agricultural productivity over the existing methods and resolved the challenges worldwide related to development of sustainable agriculture and greener ecosystems. In recent years, researchers are working on the development of different advanced microbial engineering strategies such as gene editing, CRISPR/Cas9, and RNAi to enhance the potential of microorganisms towards higher plant productivity and degradation of pollutants. The present review focused on the potential applications of genetically engineered microbial inoculants for sustainable agriculture and greener ecosystem development.

Keywords: Agricultural Productivity, Genome Editing, Green Environment, Microbial Communities, Microbial Engineering, Sustainable Ecosystem

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INTRODUCTION

Massive increase in urbanization and industrialization are the two major factors which are creating a negative impact on the ecosystem and agricultural productivity. Agricultural land is shrinking gradually due to over population, and improbable to fulfil the demand of food for an ever-growing population.[1] Currently, artificial fertilizers and pesticides are used to achieve higher yields of crops, but they are harmful to the environment and soil health. Pesticides are reported as carcinogenic and persistent in soil for a long time and cause environmental risks.[2,3] Thus, there is a need to develop new sustainable strategies for higher crop yields and greener ecosystems. Previous available reports showed that microbial engineering can play a significant role in the development of a greener environment and agriculture in a sustainable manner.[4] Microbial engineering techniques such as gene editing, CRISPR/Cas9, RNAi and others alter the genetic makeup of the microorganisms to improve their beneficial roles towards metabolization of toxic compounds that help plant growth for higher yield and to clean environment.[5] The engineering of wild microbes to produce a potent microbial inoculant offers improved crop productivity, biological control, plant growth, tolerance against biotic and abiotic stresses, nutrient uptake and increased soil fertility.[6-8] Sustainable agriculture includes different dimensions like development of agroforestry, biofertilizers to avoid chemical pesticides, production of green manure, conservation tillage, intercropping and crop rotation (Figure 1).[9,10]

Engineered microbes are reducing the use of pesticides and chemical fertilizers and can act as biopesticides and biofertilizers.[11,12] Environmental pollutants abatement using microbial remediation is a viable and efficient alternative technology over various physical and chemical methods that receive attention to manage waste from different industries.[13,14] Microbial inoculants including bacteria, fungi and algae produce novel enzymes; and secondary metabolites remove the harmful chemical with biobased processes.[15-18] Microbial engineering offers various approaches for sustainable agriculture and a greener environment by developing genetically modified microorganism through gene editing strategy that mainly include clustered CRISPR and TALEN technologies.[19]

This review focused on the management and use of microbial inoculants with improved efficacy and strategies for sustainable maintenance of the ecosystem. Overall, microbial inoculants are the formulations that can be a promising agents for environmentally friendly and sustainable agricultural practices compared to the use of conventional technologies.

Approaches for microbial engineering

Application of wild microorganisms to increase the crop productivity and in waste management face some limitations because of less efficient mechanisms to absorb toxic metals, degrade xenobiotic compounds, organic matter, heavy metals, and aromatic compounds. In view of these limitations, there is a need to explore advanced technologies that have efficient degradative properties, and are cheaper and ecofriendly. With the discovery of advanced technologies, it can be possible to understand the molecular mechanism of degradative pathways, their metabolic machinery, novel proteins, and catabolic genes to degrade the xenobiotics. So, researchers are trying to develop a new approach, i.e., genetically modified microorganisms (GMMs) that have characteristics to express desired degrading enzymes in a safer and cleaner environment. A variety of advanced molecular technologies such as molecular cloning, *in vitro* mutagenesis, gene transfer, CRISPR-Cas9 system, and RNAi etc. are used to produce genetically modified microorganisms (Figure 2). Genome editing is the change in genomic DNA via insertion, deletion of nitrogenous bases or replacement of DNA segment, resulting in either inactivation of target genes or enhanced expression of target gene at a specific target site.[21,22] In this approach, researchers are trying to insert specific novel genes that do not exist in nature and have high degradation capacity as compared to wild microbes. GMMs are more powerful in their degradative potential than wild microbes because they can easily acclimatize themselves against new pollutants. Hence, GMMs can give an alternative solution to degrade complex waste like toluenes, oil spills, halobenzoates, naphthalenes, trichloroethylene, xylenes, and

Table. Benefits of microbial engineering's green technologies compared to conventional technologies

Advantages of Green Technologies	Microbial Engineering	Conventional Technologies	References
Environmental	- Reduces reliance on synthetic chemicals. - Minimizes environmental impact and pollution.	- Often involves extensive use of synthetic fertilizers and sustainability pesticides. - Contributes to soil degradation and water pollution.	24-26
Soil health improvement	-Optimizes the microbial community, enhancing nutrient availability. -Improves soil structure and fertility.	- Reliance on chemical fertilizers may lead to imbalances and soil degradation.	
Reduced chemical dependency	- Minimizes environmental impact and pesticide resistance.	- Chemical dependency poses risks of pollution and health hazards.	
Enhanced nutrient cycling	- Promotes efficient use of organic matter. - Minimizes nutrient runoff.	- Synthetic fertilizers may lead to nutrient imbalances and runoff.	
Biological control of pests and diseases	-Uses naturally occurring microorganisms. - Minimizes environmental impact.	- Chemical pesticides may harm non-target organisms and ecosystems.	

octanes etc. instead of wild strains that degrade complex compounds very slowly.[23] The use of GMMs for waste management and sustainable agriculture offer benefits in both eco-friendly and cost-effective ways as compared to available conventional technologies (Table). GMMs reduces the need for additional fertilizers, pesticides, and herbicides which promote plant health and can increase agricultural production and soil productivity. GMMs can enhance nitrogen fixation, and nutrient uptake from the soil and could be used for the control of diseases, weeds, or pests in crop plants to make them more environmentally friendly.

Microbial engineering for a sustainable agriculture and ecosystem

Microorganisms that are manipulated for certain traits via genetic engineering are known to be genetically modified microorganisms (GMMs). In the field of agriculture and environment, microorganisms are primarily used as inoculants to offer enhanced nutrition, protection to crop plants and bioremediation of wastes etc. Several species of bacteria and other microbes can affect the growth, yield, protection of plants, and degradation of waste. Bacteria that are exploited to increase availability of nutrients to the plants for their growth, grouped as plant growth promoting rhizobacteria (PGPR).[27] Microbial populations that shield plants from pathogens, are known as biocontrol strains. Microorganisms of the phyllosphere and rhizosphere support the idea that they can be utilized in the bioremediation of soil and water pollutants.[28] Genetic manipulation can improve the microbe's potential for their possible application in diverse areas. To achieve this aim, researchers exploit genetically altered microbes with desired character. GMMs are superior in many aspects over wild types. In this section, the application of genetically engineered microbes for sustainable agriculture and a greener ecosystem is summarized.

Biopesticides

Biopesticides are modified or natural microorganisms instead of chemicals. Genetically engineered microbes can be used as environmentally friendly pesticides because it has a lesser negative impact on the ecosystem.[29] The use of biopesticides reduces the use of chemical pesticides to manage insects. Pests are the most significant threat to sustainable agriculture because they reduce the plant's yield. Pests include insects, nematodes, plants with parasitic infections, and illnesses.[30] Insect infestations hindered crop cultivation and economic development during the 20th century which employed the development of synthetic pesticides (SPs).[31] The industrial sector employs numerous synthetic pesticides, including DDT, aldicarb, fenobucarb, carbofuran, atrazine, deltamethrin (pyrethroids) and simazine (triazines). SPs, in their vast majority, induce neurotoxicity.[32] Notwithstanding their efficiency, these SPs have drawbacks that contravene the tenets of sustainability. It is critical to prioritize the utilization of biopesticides over chemical insecticides. The excessive use of synthetic pesticides can cause negative chronic health impacts such as cancer, damage to the liver, kidneys, lungs, brain and nervous system, birth defects, infertility and other reproductive problems. Agricultural workers are more exposed to pesticides with adverse health outcomes.

Biopesticides will ultimately serve as the resolution for agricultural challenges. They have numerous advantages over SPs, including enhanced health, environmental protection, and increased productivity. A wide variety of biopesticides have been developed in response to these conditions. Approximately 1400 distinct biopesticides are effectively available worldwide for the purpose of insect control.[33] The efficacy of these biopesticides is similar to synthetic pesticides. While biopesticides do possess certain advantages, the research suggests that conventional pesticides exhibit greater efficacy. The variability of biopesticides mechanisms of action increases their efficacy. The proliferation of resistant pests is a frequent consequence of the over application of synthetic pesticides; however, the development of such pests can be mitigated through the use of biopesticides which have multiple modes of action (MoA).[34] The operation of pest management (PM) systems is contingent upon the inclusion of strategically significant biopesticides. A biopesticide rich pyramid of integrated pest management (IPM) comprises over 75% of the total.[35]

Figure 1. Different approaches for development of sustainable agriculture[20]

Biopesticides are highly effective, species specific and greener approaches and they have achieved global acceptance of their use in pest management practices.[36,37] Genetic engineering is successfully utilized in developing viable alternatives against synthetic insecticides to battle against insect pests.[38] Several categories of biopesticides are known, and they account for approximately 5 percent of total pesticides produced globally, with microbial biopesticides.[39] The bacterial preparations used as biopesticides, including *Bacillus thuringiensis* (Bt), potential pathogens (*Serratia marcescens*), obligate pathogens (*B. popilliae*), and *P. aeruginosa*.[40] Bt controls 90 percent of the microbial based pesticides market.[41,42]

Biopesticides are of natural origin having active ingredients, and can target pests that are nontoxic to humans and environment.[43] Semiochemicals, secondary metabolites, etc. are a few examples of frequently used biochemical biopesticides.[13,44] Several molecules having ability to kill insects have been reported by many researchers.[45-47] Arbuscular mycorrhizal fungi (AMF) also have an important role in increasing agricultural yield.[48,49] It has been found that AMF colonization on crop plants is beneficial and provides resistance against biotic and abiotic stresses.[50,51] Microalgae strains based biopesticides have efficient anti microbial properties.[52,53] Two single celled green algae, Chlorella vulgaris and *Chlamydopodium fusiforme* and the cyanobacterium *Nostoc piscinale* are found to have biopesticide activity against pathogenic microbes.[53]

Plant growth promoting rhizobacteria (PGPR)

For the continuous supply of food to approximately 10 billion human population by 2050, the agricultural productivity must be raised to an extent of 70%.[54,55] This goal must be achieved without the expansion of agricultural land and by using a minimum amount of environmentally toxic agrochemicals.[56] PGPR

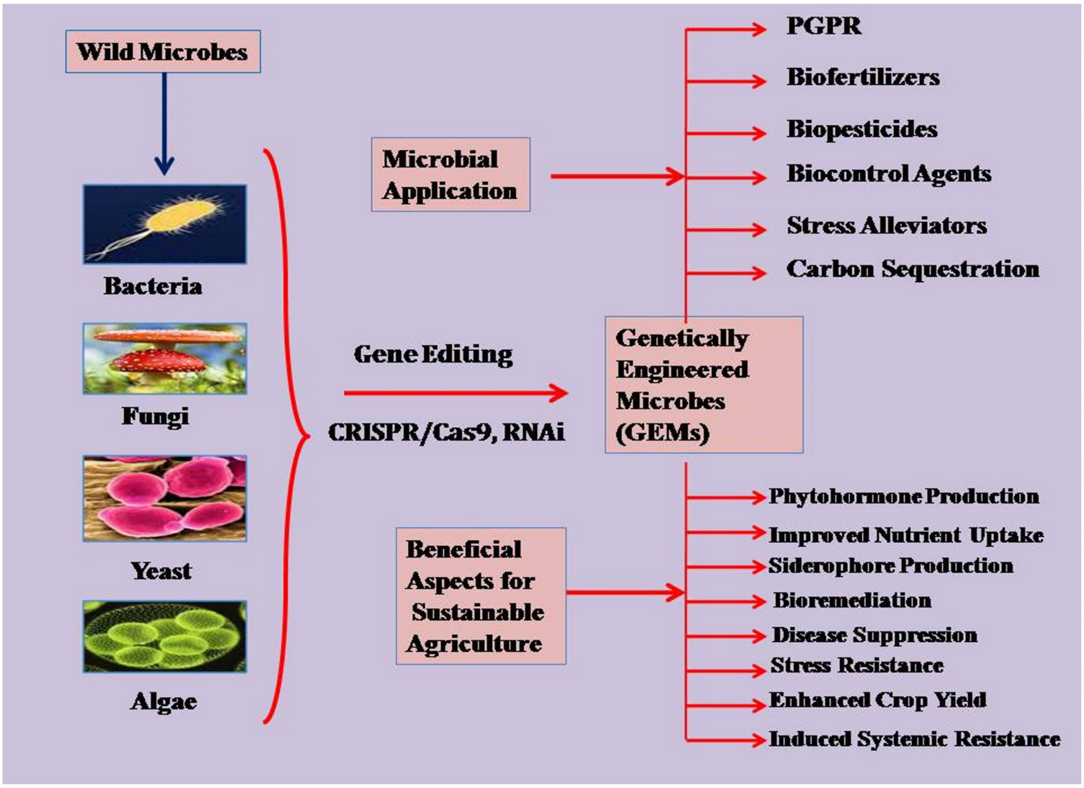

Figure 2. Genetically engineered microbes for sustainable ecosystem

reduces dependency on chemical fertilizers, and promotes sustainable agriculture.[14,57] Several microorganisms and rhizobial endophytes like *Rhizobium, Bradyrhizobium, Sinorhizobium, Ochrobactrum, Azorhizobium, Allorhizobium,* and *Mesorhizobium* are commonly used to inoculate enhanced agricultural yield.[58-61] The various mechanisms exhibited by PGPR as plant growth enhancers including potassium solubilization,[36] nitrogen fixation,[62] siderophores production,[63] phosphate solubilization,[64] nutrient fixation,[65] phosphate solubilization,[66] and suppression of plant pathogens.[67] PGPRs are also exploiting diverse methods to reduce the effect of stress (Figure 3).[68-70] During drought and high soil salt concentrations, plants experience water stress, osmotic and ionic imbalances, and increases the production of ROS.[71] PGPR aids bioremediation by breaking down xenobiotics and contaminants such as heavy metals and pesticides.

Biological control of plant's diseases

Microbial engineering may facilitate the establishment of biological control methods for plant diseases. Genetically modified microbes have the potential to produce compounds that exhibit antibacterial or antifungal characteristics, thus assisting plants in their resistance to infections.[73] By reducing the use of fungicides and antibiotics, this strategy contributes to the environmentally sustainable management of agricultural diseases.[74] Numerous biocontrol agents (BCAs) are self-sustaining and can function for prolonged durations without necessitating supplementary maintenance. *Trichoderma harzianum* Rifai, *Pochonia chlamydosporia* (Goddard) and *Paecilomyces lilacinus* (Thom) Samson have been shown to reduce the incidence of soybean root infections in Northeast China.[75] Biological control is universally recognized as a vital strategy in integrated pest management. *Trichoderma harzianum* produces antibiotics that inhibit wood decay and pathogenic fungi.[76] Fungal biocontrol strains like *Aspergillus fumigatus, A. niger, P. funiculosum,* and *P. citrinum*, etc. were reported effective against the fungus that are pathogenic in nature.[77]

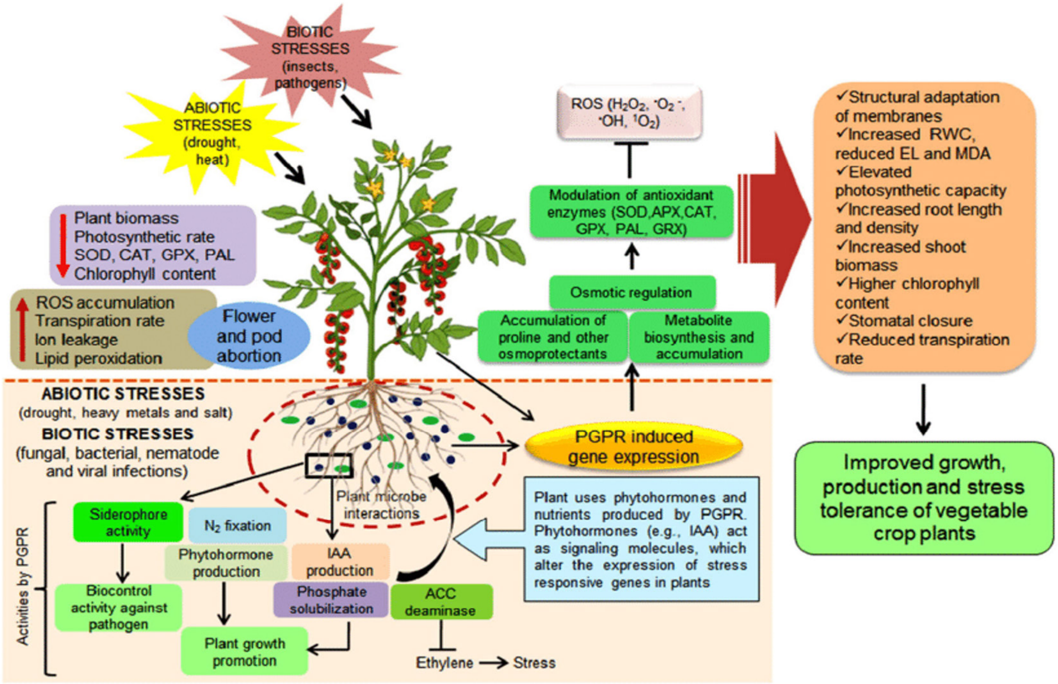

Figure 3. Mechanism of PGPR-mediated growth and stress tolerance. Plants inoculated with PGPR microbes' follow different mechanism under stress conditions such as by producing stress phytohormone indole-3-acetic acid, increasing nitrogen fixation, inducing stress-responsive gene expression[72]

Many reports are available that indicate the influence of microbes to retard the growth of potent fungal pathogens.[78] The best example of antibiosis is the use of agrocin 84 produced by *Agrobacterium radiobacter* for controlling plant disease. The genetically engineered *Pseudomonas putida* WCS358r strains, produces 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine, which cause inhibition of wheat pathogens.[79] The discovery of genome editing by using the CRISPR/Cas9 technique is a major breakthrough to apply against plant pathogens.[80,81]

Carbon sequestration

Carbon fixing microbes that have been engineered by utilizing microbial engineering can capture and store the carbon by soil microorganisms, thereby mitigating the impacts of climate change.[82] Healthy soils possess the capacity to sequester carbon, with rhizosphere microbial activity. A proper approach to manage agricultural soil, experts have recommended that soil should facilitate carbon sequestration within the range of 0.3 to 1.0 tons per hectare per year.[83] If the entire global agricultural soil area, which was 4.8 billion hectares in 2018, were transformed into grassland, considering the 2016 global anthropogenic greenhouse gas emissions of 36.2 Gton CO_2 equivalents, it is conceivable that one tonne of carbon per hectare per year.[84] However, a more cautious analysis by another set of experts suggests that cultivated soils might have the capability to sequester carbon and alleviate human-induced greenhouse gas emissions by a range of 5 to 20 percent. Implementing such a strategy may present challenges, but it highlights the potential of agricultural land management in mitigating greenhouse gas emissions. Further research, conducted by a different group, discovered that improved carbon sequestration is linked to heightened plant diversity in abandoned or degraded agricultural soils.[85] Another group proposes that the significant correlation in plant-microbes in the phyllosphere and rhizosphere supports the notion that this association can aid in the purification of air and soil pollutants.[28] Additionally, a separate study identified that mild electric fields have the potential to stimulate the decomposition of hydrocarbon pollutants in contaminated soils and enhance the activity of microorganisms associated with plants.

Biofertilizers

Chemical fertilizers precisely increases the crops yield and are hence popular throughout the world,[38] but extensive applications of such chemicals lead to irreparable damage in existing ecosytem. Biofertilizers consist of efficient genetically modified microbes, organic products and waste parts of plants which gradually increase crop yield by enhancing soil fertility. Microbes inoculated in soil provide resistance against many stresses, like hydrogen ion concentration, high moisture content, salinity, and clay content etc. GMMs offer better nutrient accessibility to crops and thus increase the growth of plants and crop yield as well. The most significant biofertilizer are symbiotic bacteria like *Rhizobium, Bradyrhizobium* and *Sinorhizobium* which forms root nodules and fix nitrogen for plants. Genetically modified biofertilizers are found to be superior in term of their activity and survival rates. GMM-based biofertilizers supply better nutrient accessibility for crops and supports agricultural practices.[86] Integrating machine learning and computational modeling provides a more accurate and efficient risks assessment associated with use of toxic compounds and give an idea about their safe utilization to minimizing the adverse effects on human health and the environment.[87]

Challenges and future outlook

Field application of PGPR was found to be a positive asset for agricultural development, but the higher crop yield achievement has been moderated due to unstable environmental conditions, and poor microbial colonization. The progress in molecular biology and genetic engineering has led to non-model microbes to be engineered for their applications. The engineered microbes are the source of beneficial microbes and are used to enhance crop productivity and environmental sustainability. There is constant debate in the application of GMM in the agricultural area. The negative aspects of using engineered microbes include the narrow perseverance of individual genotypes of microbes in the field, low survivability, gene transfer that leads to development of harmful strains and environmental threats such as increased pathogenicity and the emergence of pests. The GMMs may impose the risk to the environment upon extensive release by development of new microorganisms which are pathogenic in nature that may harm other useful microorganisms found in soil.

CONCLUSION

Microbial engineering and formulations are important for specific applications in agriculture. Researchers are showing their attention towards advancing technologies for microbial engineering. Genetic manipulation of desired traits in wild microorganisms for agricultural and environmental applications is one of the major strategies for developing efficient engineered microbes. They can be applied in plant growth promotion, environmental clean-up and others. Further research is needed for advancements in microbial engineering processes and exploration of their potentials for sustainable ecosystems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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