

MINI REVIEW ARTICLE

Insights on Engineered Microbes in Sustainable Agriculture: Biotechnological Developments and Future Prospects

Surya Sudheer^{1*}, Renu Geetha Bai², Zeba Usmani³ and Minaxi Sharma⁴

¹Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, Tartu, Estonia; ²School of Natural Sciences and Health, Tallinn University, Narva mnt 29, Tallinn 10120, Estonia; ³Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn 12612, Estonia; ⁴Department of Food Technology, ACA, Eternal University, Baru Sahib, 173001, Himachal Pradesh, India

Abstract: Background: Enhanced agricultural production is essential for increasing demand of the growing world population. At the same time, to combat the adverse effects caused by conventional agriculture practices to the environment along with the impact on human health and food security, a sustainable and healthy agricultural production needs to be practiced using beneficial microorganisms for enhanced yield. It is quite challenging because these microorganisms have rich biosynthetic repositories to produce biomolecules of interest; however, the intensive research in allied sectors and emerging genetic tools for improved microbial consortia are accepting new approaches that are helpful to farmers and agriculturists to meet the ever-increasing demand of sustainable food production. An important advancement is improved strain development *via* genetically engineered microbial systems (GEMS) as well as genetically modified microorganisms (GMOs) possessing known and upgraded functional characteristics to promote sustainable agriculture and food security. With the development of novel technologies such as DNA automated synthesis, sequencing and influential computational tools, molecular biology has entered the systems biology and synthetic biology era. More recently, CRISPR/Cas has been engineered to be an important tool in genetic engineering for various applications in the agri sector. The research in sustainable agriculture is progressing tremendously through GMOs/GEMS for their potential use in biofertilizers and as biopesticides.

Conclusion: In this review, we discuss the beneficial effects of engineered microorganisms through integrated sustainable agriculture production practices to improve the soil microbial health in order to increase crop productivity.

ARTICLE HISTORY

Received: January 30, 2020

Revised: April 05, 2020

Accepted: April 19, 2020

DOI:

10.2174/1389202921999200603165934

Keywords: Plant-microbe interactions, genetic engineering, molecular tools, sustainable agriculture, microbiome, inoculants.

1. INTRODUCTION

Sustainability is the capacity of the biosphere and human civilization to co-exist. The concept of sustainability is composed of three aspects, *i.e.* economic, environmental and social [1]. Therefore, it is rather important to think and work towards a system for conserving the resources, social supports, economic competitiveness and environmental safety. The health of the planet is mainly based on the development of an efficient and sustainable system, especially in agriculture because the soil is the base of many biological processes such as nutrient cycling, waste decomposing, biological nitrogen fixation, *etc.* which function with the assistance of soil microorganisms [2, 3]. Current agriculture practices such as using chemical fertilizers and pesticides are contributing an enormous amount of chemicals and groundwater pollution in the lands. Many synthetic fertilizers are the source of acid radicals and increase soil acidity. Soil

organisms are sensitive to chemicals added into agricultural land as fertilizers. Most of these recalcitrant compounds are absorbed by the plants and over-consuming such crops lead to systemic disorders in humans [4]. Many microorganisms present in soil could counteract the adverse effects caused by the chemical fertilizers to the environment. Therefore, reintroducing efficient strains of microorganisms in the soil is considered to play an important role in the restoring of soil ecosystems for sustainable agriculture [5]. Sustainable agriculture is a promising concept to achieve food security for the increasing world population in the circumstances of climate change. To meet the needs of food, it is expected to increase crop productivity by 70-100% which is vital for a global population exceeding 9 billion by 2050. To maintain an equilibrium between increased food production without triggering any unfavorable effect on the environment is a challenging task. One of the promising approaches to develop sustainable crop production is to enrich the beneficial microbiome associated with plants. Microbes residing in the soil system have tremendous potential to increase the growth of crops, nutrient acquisition, biotic/abiotic stress tolerance,

*Address correspondence to this author at the Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, Tartu, Estonia; Tel: +372 53652437; E-mail: soorya.m.sudheer@gmail.com

soil fertility, and disease resistance [3]. Though, with the aid of conventional agriculture farming methods that use chemical fertilizers and pesticides to increase crop productivity, air and groundwater pollution has increased at an alarming rate leading to eutrophication of water bodies. At this point, sustainable development goals (SDGs) have taken efforts to propose ways towards ensuring bio-safety through the production of “nutrient-rich high-quality food” [6]. In recent years, SDGs have channelized the innovative aspects of crop production with the aid of microbial biofertilizers as an alternative to agrochemicals. The documentation on the involvement of microbes in agriculture has emerged in the literature since 1965; however, the relationship to sustainable agriculture is yet to be revealed. To understand the role of microbes, it is necessary to study their involvement in soil conservation, crop rotation, integrated pest control and fertilizer management practices. The adoption of microbes with beneficial properties for specific tasks shall enable new research platforms for synthetic biologists to apply the new repository of tools for sustainable agriculture. Moreover, with the development of systems biology, and synthetic biology tools like (re)engineering genomes through homologous recombination systems, CRISPR/Cas9 showed great potential in promoting the efficiency of the microbial inoculants used in sustainable agriculture practices and their interaction with plants and other functional groups of soil microorganisms. Given that the recent developments in molecular tools gears for both bacterial and fungal systems, the advance of novel technologies as one of the next edges in biotechnology.

2. PLANTS VS SOIL MICROBIOME

Soil is a reservoir of widespread microbial communities that are vital for various soil functions and are quite complex. It is essential to understand the behavior of these microbial communities in soil and how they work together in response to changing environmental conditions. Since the last two decades, researchers have focused on the effect of biodiversity loss on ecosystem functioning and how it varies among the functional traits of species in the above ground and below ground soil. It is commendable that research on soil biodiversity is stepping into a new phase that scientists and policymakers are now more aware of the importance of soil biodiversity and its beneficial side to human society and ecosystem [7]. Soil microbes play a central role in the formation and improvement of soil fertility, farmland material circulation, enhancing stress resistance in plants, resistance towards soil-borne pathogens, degradation and detoxification of heavy metals in soil [8]. Plants have evolved to cope with environmental stress in association with soil microorganisms and such plant-associated microorganisms are called plant microbiota and also referred to as plant holobiont [9]. Recent studies have shown that plant-soil microbiome interactions can be complex and varies from plant species. Soil microbiota interacts with the plants either directly or indirectly improving the health and fitness of the plants [10]. Healthy plant-microbial interactions support plants to manage with varying stress conditions and diseases, it improves the exchange of mineral substances such as nitrogen or phosphate, and act as biocontrol agents to prevent pathogen attack in plants [11, 12].

Microbiome in the soil represents all types of microbes living in the soil ecosystems that include bacteria, fungi, archaea and protozoa. Many of them are critical performers in augmenting soil fertility and crop yield. It is estimated that 10^9 microbial cells/g of soil are documented and display a great level of variety. About 10% of microbes living in plant-influenced areas are capable of growing in standard culture media, whereas the others are uncultivable microbes, but recognizable using molecular techniques [13]. Microbes foster agriculture productivity by aiding and controlling the availability of supplements to plants and by promoting tolerance towards environmental stress conditions. Plant associated microbiomes are expected to increase plant adaptability and high-yield in agriculture. Plant-microbe interaction is one of the significant, exciting areas of research since the evolution of crop improvement manoeuvres. Understanding the role of plant microbiomes and their responses to the changing environmental conditions is thus vital in the development of a sustainable crop improvement concept. In a beneficial plant-microbial interaction, mutual benefits exist for the interacting organisms. As an outcome of photosynthesis, more organic biomasses are released into the soil *via* root exudates of plants and then used by soil microorganisms as a substrate to grow. These microorganisms, in turn, improve plant growth *via* different mechanisms to facilitate plant nutrition, resistance to pathogenesis, and many more vital functions. Members of the plant microbiota are metabolically adapted for the utilization of plant-derived carbon compounds. Apart from the utilization of rhizodeposits and low molecular-weight carbon sources, the metabolism of one-carbon compounds and plant cell wall components are frequently found in plant-associated bacteria [14]. Different modes of interaction occur between soil microorganisms and plant roots. Those microorganisms living close to the root, and utilizing N_2 and C metabolites release from the root are called rhizosphere microbes or rhizomicrobiome. Some microorganisms colonize on the root surface of plants, whereas, few microorganisms colonize inside the intracellular spaces of root tissue called endophytes. The specialized microbes which live inside the plants *via* specialized root structures or nodules are called symbiotic microbes (Fig. 1).

Rhizomicrobiome fosters the yield and productivity of crops by contributing in different ways, such as improving soil fertility and texture, nutrient acquisition, secretion of intra and extracellular molecules like antibiotics, hormones, signalling molecules and secondary metabolites [15]. It is necessary to keep a better understanding of the possible mechanisms and pathways involved in designing effective strategies to combat the increasing food demand in the world without damaging the ecosystem as opposed to conventional agricultural practices. Though the plant microbiome is recognized as a crucial potential player in microbial diversity, the associated plant species and their relation with bacterial communities are yet to be studied. The developments involved in biological technologies make it feasible to use microbes and their metabolites enrich the uptake of plant nutrients, crop yield, pest control, and alleviate stress responses in the plants. To have in-depth knowledge and understanding of the future possibilities of microbial technology, their efficacy and reliability in the real-world conditions have to be improved. Moreover, the lack of suitable methodology has in-

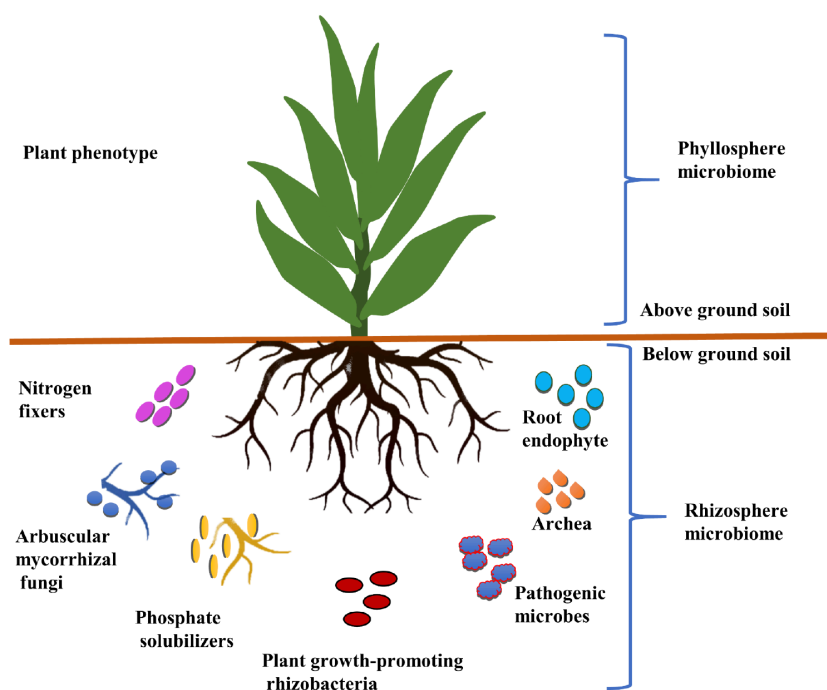


Fig. (1). An illustration of various microbes involved in plant-microbial interaction. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

hibited the developments and understanding of the wide-ranging mechanisms in the rhizosphere involving plant-microbial relations.

3. HOW PLANT-MICROBIAL NETWORKS WORK IN THE SOIL?

Plants are associated with a range of other organisms, which includes epiphytes (living on plant surfaces), endophytes (living within plant cells), soil and rhizosphere microbes, which are living in association with subsurface plant organs and root-soil interfaces [16]. The rhizosphere zone embodies the most diverse habitat where plants and microbes interact with each other and are central to ecosystem functioning. Plant-microbe interaction benefits all plants in the ecosystem and increases productivity. Different factors such as the interaction between microbes, secretion of exudates by plant root, genetic exchange between uni/multicellular organisms, nutrient transformation and exchange favor the subsurface world [17]. Understanding the complete functioning of rhizosphere microbes is important to manage the ecosystem and harness its potential benefits effectively. A major mechanism to improve agriculture or forest sustainability, refining water quality, plant productivity, climate alteration and biodiversity conservation is by manipulating the rhizosphere.

Understanding the mechanism of how microbial populations respond and recuperate from instabilities such as a pathogenic attack, climate change, drought, *etc.* is a real challenge for the researchers. By reports, individual microbial communities react in a different way to climatic circumstances, *e.g.* soil fungi are more resistant to drought conditions but comparatively less resistant than bacteria [18]. Furthermore, the regaining of fungi, as well as bacteria towards stress condition, is governed by plants and its response to

stress conditions. Though some of the previous studies have shown the impacts of climate change and other disturbances on soil microbial communities by focussing on single properties of soil microbial communities and their functioning, there is a gap in determining the multitude of direct and indirect interactions that occur between the microbial networks that co-exist in the soil [19]. De Vries and coworkers reported an insightful study showing that drought has sustained effect on the bacterial communities and their co-occurrence network by changing the vegetation composition and subsequent reduction in soil moisture, which reveals the potential long term consequences on the above and below ground microbial communities to withstand future disturbances [18].

Plant associated microorganisms such as prokaryotic bacteria and eukaryotic fungi thrive in a variety of living habitats and interact with plants *via* symbiotic or saprophytic relation. Most of the microbial population remains in the rhizosphere zone, but some are able to penetrate and live inside plant tissues called 'endophytes' [20]. The endophytes colonize in different parts of plants and different compartments of plant apoplast. Most of the endophytes can escape from the plant immunity response and colonize inside plant cells affecting the growth of the plants and reactions to pathogens, herbivores, and environmental fluctuations. Most endophytes are uncultivable, as a reason the diversity analysis and molecular interactions with plants need to be analyzed by molecular approaches.

The relation between soil microbes and plants works on positive or negative feedback mechanisms among each other based on the chemical environment. For example, to face the stress conditions, plants communicate with the microbial community through an intricate series of any trophic cascades [21]. Plant-microbe communications are generally controlled through quorum sensing (QS) *via* a population-

dependent mode [22]. In QS, microbes produce a low-molecular-mass signalling molecule which can be recognized by microbial cells and allows the whole population to initiate an intensive action once a critical concentration has been achieved. Bacterial communications in various Gram-negative bacteria produce common signal molecules, *i.e.*, Nacylhomoserine lactones (N-AHLs). The study conducted by Crepin and coworkers reveals that a rhizosphere bacterium, *Rhodococcus erythropolis* was capable of catabolizing the N-AHLs created by a pathogenic bacterium, *Pectobacterium atrosepticum*, consequently reducing its virulence, which is a tritrophic chemical interaction. Pathogens utilize QS consuming N-AHLs to create microcolonies (also biofilm) in the rhizosphere to impose pathogenicity in host organisms [23]. One of the main challenges faced by researchers is profiling of an intelligent group of procedures where the uncultivable microorganisms (*e.g. Tannerella forsythia, Abiotrophia spp.*) dominates some of the microbial communities.

In the current situation of environmental/climatic changes, advancements in molecular methods are quite promising, which lead to the development in the studies of soil microbial diversity and plant-microbe interactions. For example, to study the stress responses in plant-microbe interaction, in the crops, it is possible to expand the capability of soil microbes for stress alleviation [24]. Different abiotic and biotic stress factors, such as contamination, diseases, nutrient deficits, drought, pests, and salinity, *etc.* change plant-microbe relations in the rhizosphere. Modifying the crops or microbes against any of these factors would offer a better possibility for crop improvement.

4. APPLICATION OF MICROBIAL INOCULANTS IN AGRO-ECOSYSTEM

Microbial inoculants are described as plant stimulators because of their beneficial functions in agricultural crop productivity. The host plant microbiome interaction is crucial for plant growth because microbes could affect the growth of plants and development at different stages of growth, *i.e.*, germination to flowering [25]. Upon application, in the rhizosphere soil, microbial inoculants stimulate the normal processes to enrich nutrient uptake and productivity, abiotic stress tolerance, and quality of crops (Fig. 2). These microbial inoculants are frequently counted in agricultural management carry-outs intended at reducing chemical contributions, increasing efficiency and improving the natural stability of agro-ecosystems [26].

Soil inoculants are applied in the soil by combining diverse classes of soil organisms within single inoculant so that they could possibly take benefit of numerous plant growth encouraging mechanisms. But identifying the mechanisms performed by each class of organisms in the soil will be difficult to predict [27]. A certain group of bacteria collected and isolated from soil have the property that permits them to employ favorable properties on plants but some of them even upon inoculation to soil fail to show expected results. For example, *Azospirillum*, *Rhizobia* and *Agrobacterium* released into the field as a seed inoculant often increased crop yield whereas *Pseudomonas* species failed to give expected results. It is rather complicated that even upon

the release of genetically altered microbial inoculants, it is difficult to predict the bacterial existence and persistence and also the expression of improvements in their traits. It is possible these days to observe the altered inoculant bacteria subsequent to their introduction in field ecological unit and to evaluate their influence on the local microflora. Similarly, local environmental influences play a substantial role in defining the endurance and perseverance of bacteria when released in fields. Temporary shifts in favor of the new bacteria and disfavor of some existing populations of the bacteria and fungi in the plant rhizosphere might happen with some inoculant release. The changes observed were, however, less important than those observed under usual agricultural practices. Intragenic and intergenic gene transfer among soil bacteria was reported in some studies [28].

The utilization of microbial-based crop improvements is growing globally, especially in developing countries like Asia and Africa, where multi-strain developed from rhizosphere soil has been practiced with a success rate of 10% increase in the grain yield [29, 30]. One of the challenging aspects during the microbial inoculants introduction in agriculture structure is overcoming the colonization or conservation of new inoculants in the rhizosphere. Numerous studies have shown successful microbial colonization in the soil, whereas if it comes to agricultural context, the yield often shows inconstant or temperate results with a fast decline in the inoculant number and its activity in the soil [31]. The main reasons considered for the decline in the inoculant population maybe competition with indigenous soil microorganisms, changes in growth conditions such as humidity, pH, texture, and temperature. Agriculture practices like tilling and heavy use of agrochemicals also impact the efficiency of microbial inoculants. Another consistent factor is the choice of a host plant and its association with the inoculant. But this selection may vary based on the immune system of the host plant, plant root exudates, and the indigenous endophytes present in the plant tissue [32].

The microbes that live in the rhizosphere soil interact with plants and bring beneficial effect to the host plants. In plant-microbial interactions, plants release root exudates to attract mutualistic microbes which could improve plant functions such as nutrient uptake, yield, stress resistance, *etc.* Using various molecular techniques, plant-microbial interactions could be well studied and beneficial strains of microbes can be selected for genetic plant functions such as nutrient uptake, yield, stress resistance, *etc.* Using various molecular techniques, plant-microbial interactions could be well studied and beneficial strains of microbes can be selected for genetic modifications. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) and RNAi (RNA interference) are efficient methods for gene editing to manipulate improved strains of microbes of interest. Genetically modified microbial inoculants are then applied in agro-ecosystem to achieve a sustainable increase in plant/crop productivity.

4.1. Microbial Inoculants as Biofertilizers

For attaining the food requirements of the increasing population, maintaining soil fertility is extremely necessary. Biofertilizers are one of the best alternatives to meet this

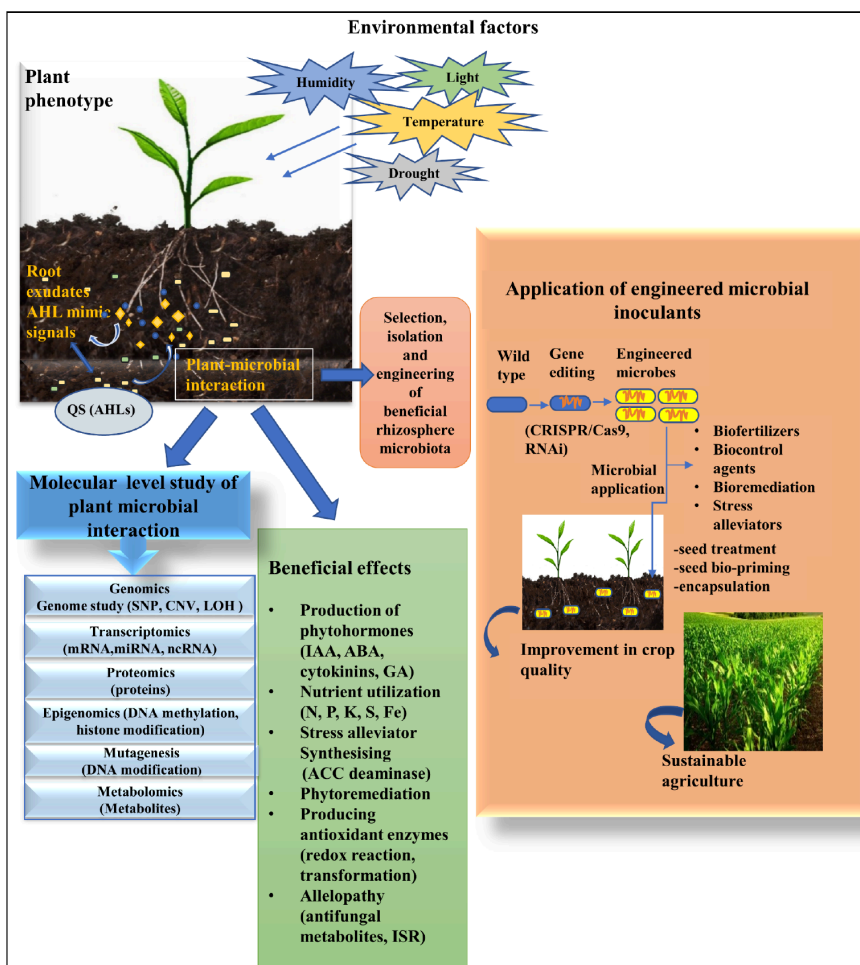


Fig. (2). Plant-microbial interactions and sustainable agriculture applications. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

requirement, as they are made of beneficial microbes which can increase plant productivity and escalate food production without affecting environmental stability. The microorganisms present in the biofertilizers supply nutrients to plants that are already present in the field. Nitrogen-fixing bacteria, mycorrhizal fungi, phosphorus, potassium and sulfur solubilizers are well-studied examples of rhizosphere microorganisms used as biofertilizers [33-35]. Microbial inoculants are applied in the form of liquids or in dry formulations. In order to have optimum functionality of microbial inoculants in the field, a larger amount of inoculum is needed, which is practically not feasible [36]. During the inoculation of biofertilizer to cropland, microorganisms used as biofertilizer should reach and colonize around the rhizosphere zone of the plant and initialize a plant-microbe interaction and encourage the plant growth by direct and indirect mechanisms. Based on the mechanisms of action, microorganisms used as biofertilizers are classified into symbiotic biofertilizers and asymbiotic biofertilizers. Symbiotic microorganisms always keep a symbiotic association with the roots of host plants, and form specialized root or structures in and around the plant root. Primary symbiotic organisms are nitrogen-fixing rhizobacteria and arbuscular mycorrhizal fungi (AMF) [37]. Nitrogen fixing bacteria, such as *Rhizobia* species generally inhabit in the legume root nodules. Mycorrhizal symbiosis with plant roots is relative, and their interaction favours N, P, and water

uptake by the plant. Ectomycorrhiza and endomycorrhiza are the two main types of mycorrhizal association with plants. Ectomycorrhizal fungi colonize plant roots as a net in the outer cell wall layers of plant roots without invading into the plant cells (e.g. species from the phyla Basidiomycota and Ascomycota). One of the endomycorrhizal fungi, arbuscular mycorrhiza, colonizes the root cortex to form a mycelial network and form vesicles and arbuscules in root cells having adsorptive and storage functions. Possibly the most common AMF in natural and anthropogenic habitats is *Glomeraceae* species in genera *Glomus*, *Rhizophagus* and *Funneliformis* (earlier all in *Glomus*) [38]. Non-symbiotic biofertilizers such as plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Actinobacter*, etc. [39] reside outside the plant roots and colonize in the rhizosphere area. PGPR species have been the most studied species, and different authors have proposed various definitions to organize the concept of biofertilizer viz, PGPB, PSHB. Plant growth-promoting bacteria (PGPB) are microbes, directly influencing the plant growth by improving the mineral nutrient uptake as well as through the synthesis of phytohormones [40]. Some PGPR species are highly selective and impact selected organisms which again causes inconsistency of quality and efficiency in field conditions [41]. Plant stress homeostasis-regulating bacteria (PSHB) are the microorganisms that support plants under

stress conditions [40]. Some biofertilizers are reported to increase the nutritional properties of vegetables by enhancing the metabolic contents in those plants which contribute to antioxidant potentials in human health. For example, the amount of total phenolic compounds, carotenoid contents and anthocyanins of lettuce has been increased upon inoculation with *Glomus fasciculatum* and *Azotobacter chroococcum* [42]. Similarly, soybean seedlings inoculated using *Rhizobacteria* showed an increase of 75% in the biosynthesis of phenolic acid [43]. Based on the treatment methods and storage conditions, viability of microbes used in the inoculated seeds vary and it will affect the success of inoculation in the field.

4.2. Microbial Inoculants as Biocontrol Agents

Many bacteria, fungi, and actinobacteria are used as biocontrol agents to protect plants from harmful pathogens and perform antibacterial and antifungal activities. A variety of microbial inoculant formulations are offered in the market which have potential applications in agriculture as well as horticulture crops. These microbial inoculants work by either releasing hydrolytic enzymes (extracellular), competing for the nutrients and the secondary metabolites that are detrimental to the plant pathogens at lower concentration [44]. Some of the microbial inoculants are reported to have herbicidal activity. *Colletotrichum coccodes*, a mycoherbicide of velvetleaf and mycoherbicides of *Striga* are few examples that have already been reported by researchers [45, 46]. Another popular example is the antibiotics produced by *Trichoderma harzianum*, which inhibits wood decay and pathogenic fungi [47].

Fungal biocontrol agents such as *Aspergillus niger*, *A. fumigatus*, *Penicillium aurantiogriseum*, *P. citrinum*, *P. funiculosum*, and *Trichoderma koningii* were found active against the plant pathogenic fungi *Phytophthora infestans* [48]. Many researchers have reported the efficacy of *Amphibacillus xylanus*, *Bacillus amyloliquefaciens*, *Sporolactobacillus inulinus* and *Microbacterium oleovorans* in growth inhibition towards fungal pathogens [4]. *Mitsuraria* sp. provided remarkable biocontrol effect on the bacterial leaf spot diseases [49]. *Pseudomonas* spp. exhibited a biocontrol outcome on *Fusarium* wilts [49]. *Bacillus* spp. were evaluated as effective in the regulation of microbial diseases in many plants by biological control as they are capable of producing volatile inhibitory substances [49]. *Rhizobia* sp. demonstrated promising effects as an effective biocontrol agent for *Pythium* disease [50].

4.3. Microbial Inoculants Designed for Biotic and Abiotic Stress Tolerance

Due to climate change and natural and anthropogenic factors, crops are facing increased stress, which leads to a decrease in crop productivity. Though many trials and studies have been conducted to develop strategies to deal with abiotic stress, still a permanent solution seems to be a challenging task. Microorganisms have been found to favor plants under such conditions and could help in fighting against stress *via* some direct-indirect mechanisms. Stress can be categorized into biotic and abiotic. Biotic stress is caused due to pests and plant pathogens (bacteria, fungi,

nematodes, insects, and viruses), whereas abiotic stress could be caused by drought, flooding, gases, heavy metals, salinity, temperature, and nutrient quantity. These stress conditions could result in a yield reduction, based on the plant factors and soil types and also the ecology and evolution of plant-soil microbe interactions [51]. There will be an imbalance in nutritive elements, hormones, physiological disorders (abscission, epinasty, and senescence), and susceptibility to ailments [52].

PGPRs are efficient bacteria that could work on some soil types and help plant growth *via* direct or indirect mechanisms. In the direct mechanism, the nitrogen is fixed *via* biological nitrogen fixation. Phytohormones like IAA (indole-3-acetic acid), iron and phosphates solubilized by bacterial siderophores are provided to the plants. In the indirect mechanism, PGPR releases enzymes such as bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that stimulates physiological changes at the molecular level. ACC deaminase directly controls the plant ethylene production, resulting in altered plant growth and progress. ACC deaminase producing bacterial strains assist plants in eliminating the adverse effects produced by stress. Plants treated by bacteria having ACC deaminase were found to have widespread root growth owing to fewer quantities of ethylene. Like this, plants may fight several sources of stress. Recently, PGPR with ACC deaminase activity was used to enhance the plant growth under nutrient deficiency, heavy metal stress, salinity and drought [53-55]. For example, chickpea plants circumvented drought stress with the help of PGPR bacteria *Pseudomonas putida* -MTCC5279 by the differential expression of genes involved in ethylene biosynthesis (ACS and ACO), salicylic acid (PR1), and jasmonate (MYC2) signalling. *P. putida* exhibited drought tolerance by modifying membrane integrity, ROS scavenging ability and osmolyte accumulation [56]. Similarly, ACC deaminase making strain of *P. fluorescens* REN1 improved root elongation in rice plant under constant flooding situations [57]. Inoculation of the tomato plants subjected to low temperatures with *P. frederiksbergensis* OS261 and *P. vancouverensis* OB155 increased the expression of antioxidant activity and cold acclimation genes in leaf tissues [58]. Similarly, AMF is found to bring tolerance to stresses like drought and salinity. AMF have a well known function in improving plant tolerance to pathogens and abiotic stress, therefore, AMF inocula are highly preferred in restoring the agriculture and forest lands [59, 60].

5. MICROBIAL INOCULANT DELIVERY IN THE FIELD

Another important factor that must be given consideration is the inoculant delivery in agriculture fields. Upon direct addition of microbial inoculants to the field, 90% inoculants could be gone during application in the field and it imposes a considerable cost to the farming arrangement and labor cost. Therefore, an effective method of inoculum dispersion into the field is important which could allow controlled release and longer effective in the agriculture field. It will ensure more success for the microbe mediated improvement of crops. Seed treatment, seed bio-priming, encapsulation methods and root dip are the main delivery systems already in practice [32, 61]. For example, PGPR is de-

livered through several means such as seed treatment, root dip, soil application, and irrigation based on the survival in nature and mode of infection of the pathogen. It is anticipated that a model microbial inoculant formulation facilitates the delivery of the living biocontrol agents in their viable state, at the right place, and at the right time [61]. In seed treatment, an inert carrier (such as gum arabic and xanthan gum) facilitates product adherence to seeds by mixing seeds with the formulated products [62]. In seed priming, the seeds are mixed with an organic carrier and then the moisture content is brought to a level just below what is required for seed treatment. It was tested to deliver *T. harzianum* to control Pythium induced damping-off on cucumber [63]. In microbial seed bio-priming techniques, a substantial increase in the microbial groups applied to seed exteriors are observed [5] which could result in early activation of the priming inoculants beforehand networking with spermosphere (*i.e.* seed surrounding zone) pathogens [36]. In encapsulation technique a specialized seed-coating (alginate microbeads) process is used which involves enveloping the seed with microbes, and possibly a few other components such as pesticides or micronutrients, in a gelatinous or polymer gel matrix, thereby prolonging the survival of microbial inoculants on seed [64]. Significant plant growth was observed when *Bacillus subtilis* CC-pg104 and *Pseudomonas putida* CC-FR2-4 encapsulated in alginate complemented with humic acid was used to inoculate *Lactuca sativa* L. [65]. Micro-encapsulation and micro-composites of beneficial microbes with bentonite and alginate are verified to raise the effectiveness of microbial inoculants in an agricultural setting [66, 67].

6. ROLE OF GENETIC ENGINEERING AND MOLECULAR METHODS IN PLANT-MICROBIAL INTERACTION

Meeting agriculture productivity without losing the quality of agricultural land is more promising by the application of microbial inoculants. But at the same time lack of appropriate methods to understand the mechanisms of underlying plant-microbe interaction in the rhizosphere is another constraint. Taking this into consideration, researchers have contributed valuable time for developing and engineering microbial inoculants that could apply in the agriculture land as biopesticides, bioherbicides, biocontrol agents and biofertilizers. Microbial inoculants are destined to be eco-friendly and sustainable plant nutrient transporters. They have the capability to minimize chemical input impacts and accordingly intensify the quality and quantity of farm products. Use of microbial inoculants reduces the application of chemical fertilizers. Microbial inoculants will be a promising environmentally sustainable approach for the future green revolution, since they are connected closely with plant growth, health, and productivity [32].

Genetic engineering has already advanced that engineered microbes which have been used from biosynthesis to bioremediation. The technologies for the production as well as the application of microbial inocula are under constant progress and improvement. Several PGPR species are already used worldwide as biofertilizers, contributing to plant growth-promoting mechanisms; hence it is important in forestry and sustainable agriculture productivity [68]. Soil mi-

crobial population is multifaceted, active and differs in structure between different sections and levels, this creates an actual challenge for researchers. A critical problem to face such research is sampling entities. The major concerns include replicating numbers, sample size, type of sampling (randomized or regular intervals), microsite variation and spatial scaling. Even rhizospheric soil, which is mostly researched, is also practically very tough to precisely define. Though, to explain a more generalized response, the side-distance outcome on bulk soil is more reliable. Time-course reports are also essential in monitoring inoculation results according to the buffering capability of the agro-ecosystem. However, the methods used to examine soil microbial groups at functional and taxonomic levels are difficult and restrict the exhaustive samplings. Usually, for culture-dependent approaches, the study is limited to restricted samples. Whereas, the culture-independent approaches usually do not authorize the definite identification of taxonomic sets. Also, the bias brought by PCR amplification and DNA extraction, the culture-independent approaches also represent some inherent restrictions [69].

A plethora of molecular techniques have been developed to learn the variety of microorganisms occupying the rhizosphere zone and how effectively, they interact with each other including the influence of global climate change on soil microbiome diversity (Fig. 2). Another fact to notice is that with these molecular techniques, the total soil microbiome together with the unculturable microbes can be detected using molecular markers. The functional capability and phylogenetic identity of microbes could be characterized by DNA and the gene expression, in a given state could be studied by referring to the RNA. Metagenomics studies having been used for many years to study environmental samples. Metagenomic study involves DNA isolation and cloning, which comprised of some genes and operons which will be exposed to diverse techniques by cloning approaches, high throughput sequencing, PCR amplification, or microarray hybridization. The creation of metagenomic libraries which could be screened for functional and structural genes or for phenotypic characters linked to proteins, together with enzymes, and also secondary metabolites profiling [24]. Different PCR derived quantitative methods make it easier to amplify microbial DNA take out from soil samples and resulted in easier decoding of microbial diversity. Researchers prefer 16S (for bacteria) and 18S (for fungi) small ribosomal subunit sequences as microbial community target molecular markers. The sequence investigation of this cloned 16S/18S rRNA gene is the base to compare, microbial richness, evenness, composition, and structure of microbial groups. The PCR products called amplicons, having identical, or analogous variable region is operational taxonomic units (OTUs). The amplicons are later assessed by molecular typing approaches (which permits a specific molecular fingerprint aimed at a target microbial community structure), cloning and by sequencing methods. Nowadays high throughput next-generation sequencing procedures such as pyrosequencing allows assessing the sequence of the amplicons directly so that taxonomic character from the phylum to genus level of microbial groups in various soils and biomes can be possible. On the other hand “single molecule real-time sequencing” (SMRT), a third-generation sequencing technology does not

even need a PCR analysis since it is based on a single DNA molecule. Functional gene microarray-based methods such as PhytoChip and GeoChip are two promising approaches to monitor the richness of specific taxonomic set in the communities. The functional gene arrays are developed to measure the activity of definite functional microbial actions. One of the modern analytical technique proposed to study the rhizosphere colonization dynamics is the fluorescence *in situ* hybridization (FISH), in which DNA/RNA probes tag homologous sequence microbes and enable localization.

Plant microbial interaction and sustainable agricultural applications deal with either the usage of microbial groups or a precise modification of microbe or plant. Genetic modification method and gene silencing were extensively used to learn gene functions and trait improvement. Another promising approach called transgenic technology helps to get a faster outcome through the incorporation of extraneous genetic material that confines its extensive usage due to regulatory matters [70]. In this regard, gene editing is getting much attention so that editing genomes in a specific manner without the incorporation of a foreign gene. In this technique engineered endonucleases create a double-strand break (DSB) that endure DNA repair through endogenous mechanisms, thus create diverse mutations *via* two main pathways *i.e.* homology-directed repair (HDR) and non-homologous end-joining (NHEJ) [71].

Recent development has also exploited CRISPR–Cas to enhance the efficiency of genome editing in microbial systems to be used in agriculture and to perform strategic genetic knock-in to activate silent biosynthetic gene clusters and improved metabolic output [72]. There are several ways by which targeted genetic modification could be achieved. The most used are three meganucleases, which are CRISPR/Cas (CRISPR-associated) system, transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) [73]. The genome editing capability of ZNF/TALEN is ruled by factors like the DNA-binding affinity, TALE proteins and specificity of the gathered zinc-finger. Whereas, CRISPR/Cas structures are more efficient, versatile, cost-effective and specific. It has been used as the most attractive gene-editing tool to study the genetic and molecular aspects of plant microbial interactions [74]. Through CRISPR/Cas, a Type II bacterial immune system has been seen in quite a few prokaryotes. About 40 % bacteria and most archaea species are reported to have CRISPR loci [75]. An organized idea of the plant-microbial communications will permit the utilization of proper molecular tools to boost the capacity of plants or microbes for agronomic trait enhancement [76].

Synthetic biology is a rapidly growing field of science involves redesigning of organisms for useful purposes by engineering them to have new abilities. Synthetic biology promises an increase in crop productivity and sustainability by modifying microbial genomes involved in crop improvement. It can provide various tools to address many of the challenges faced in agriculture and benefits the global economy. To provide the necessary foundation for systems and synthetic biology research of microbes in agriculture applications and/or interactions, multidisciplinary characterisation is necessary including DNA barcoding and recently

multivariate modular metabolic engineering techniques for strain improvement. This technique has huge potential for enhanced products from microbial sources to be used in sustainable agri-biotech that also helps in developing an optimised strain for improved agriculture practices. Application of CRISPR/cas9 technology is ubiquitous across synthetic biology. One of the key applications of CRISPR/Cas9 to agriculture is the potential for nuclease-based gene drives to eliminate pest species [77]. CRISPR-based different tools are used in chromatin engineering, gene regulation, single base editing, epigenetic editing, imaging, *etc.* [78, 79]. Screening of the target gene from a large population is possible these days through different CRISPR-based screening techniques such as barcoding, gene tagging, nucleic acid detection, and lineage tracing, along with functional-specific genomic library [76]. For successful agriculture production, certain aspects to be considered such as increase the inoculum production, create quality control protocols, minimizing the variability in field trials, and to create specific normative to each inoculant category and its application [24].

Next-generation sequencing studies have provided deep insight into the community composition of the above and below ground compartments of various host plants ranging from tree species to crop plants. From some of the studies, it was confirmed that the bacterial plant microbiota is composed of only a few dominant phyla of *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* [80]. García-Salamanca and co-authors revealed that bacteria belonging to the phyla *Bacteroidetes*, *Acidobacteria*, and *Proteobacteria* were predominant in the bulk soil, whereas, *Gammaproteobacteria* found prevalent in the rhizosphere in the study of microbial groups that inhabit maize plants cultivated in carbonate-rich soil with pH 8.5, *via* culture-independent PCR-based approaches [81]. This indicates the variability in the classes of organisms that adapt to the rhizosphere soil habitats. Bacteria that live on the root surface can effectively modify root phytohormone and thereby promote the nutrient availability and growth in plant. In case of the mycorrhizal fungi, they change root aquaporin gene expression, and critically enhance the surface area, so that the plant root structures take up nutrients and water. Muller and Sachs have developed a promising strategy for the selection and introduction of beneficial indigenous inoculant based on breeding methods [82]. According to their method individual plants displaying best performance, such as growth, disease resistance, or drought resistance are identified and screened for microbes harboring a phenotype of interest and isolated either from the rhizosphere or root compartments. Harmful pathogens were removed and the remaining potential isolates were used alone or combined as composite microbial inoculum. For different crop varieties, mixed microbial inoculants can be used, for this microbial consortia need to be crop-optimized over sequential inoculation in order to intensify microbial colonization as well as the plant beneficial properties. In comparison to single isolates, microbial consortia showed more efficiencies, higher chances of existence and activities in the roots of the crops [3].

RNA interference (RNAi) is a recent genetic engineering tool to amend the genes at the expression level. RNAi is a vital procedure in plants in order to resist pathogens. In

RNAi, plants inhibit both the transcriptional and post-transcriptional gene expression by three diverse sets of proteins. Though, pathogens activate an anti-silencing mechanism to block host RNAi *via* the expression of some suppressors. The interruption of the silencing step is a virulence policy of pathogens to endorse infection in the conquered hosts [83]. In agriculture, the efficacy of RNAi anti-pathogen has been proved. In a study Ganbaatar and co-authors utilized *Escherichia coli* strain holding RNAi sequences to eliminate a corn pathogen, *Mythimna separata*. In this case, genetically modified microorganisms did not destroy the pathogen straight away instead transferred the dsRNA to silence the targeted genes in the pathogen [84]. It is a promising tactic to engineer beneficial microbes and intensify the plant resistance to specific pathogens. Moreover, it could help to get knowledge of the gene expression and functions and also manipulating the genes to get desired genotype and phenotypes *viz.*, defense against invading pathogens, nutrient acquisition and mobilization [32].

When considering plant-microbial interaction, host genetics has a projecting role in creating the microbiome composition [85]. It is reported that many of the plant genes and its function are correlated with plant microbiota and its surrounding environmental conditions [86]. Quantitative genetic tools like QTL (quantitative trait loci) mapping are useful in allowing the identification of gene loci underlying significant biological characters of any organism [87]. These information improved plant varieties through genetic engineering and further plant breeding methods. As soon as QTL or genetic characteristics of crops, which controls the communications between crop and helpful microbiomes, are recognised they are utilized to produce novel and better-quality crop variations which can attract and connect advantageous indigenous microbiota [32]. The CRISPR/Cas9 technology holds great potential in improving the capability of plants to favorably recruit helpful microbiota [88]. An analogous method can be used to deploy the primary colonization *via* hub microbiota to ultimately form the fundamental and complete plant microbiomes in probable manners. Moreover, a combination of conventional breeding methods with genetic engineering and omics technologies has great potential to enhance crop improvement. New findings in molecular biology and genomics have already offered tools for enhancing the efficiency of conventional plant breeding and genetic engineering of crops. But some researchers claim that molecular approaches to conventional breeding are safer and superior than genetic engineering [89, 90]. Under certain circumstances, conventional breeding could not achieve some traits upon incorporation with modern molecular tools. As an example, incorporation of a natural insecticide from another species into a particular crop plant, like Cry proteins from *Bacillus thuringiensis*. Modification of the plant microbial interaction system against microbial pathogens may be an interesting to improv the disease resistance in agricultural crops. Modern bioengineering technologies are helpful on enhancing plant resistance against pathogens by using genetic engineering [91] and thus increased agricultural productivity. The most important genes for this purpose are those encoding chitinases, glucanases, peroxidases and antifungal proteins from *Trichoderma* species [92].

7. FUTURE PERSPECTIVES

Engineering the microbes could effectively enhance the crop productivity in the fastest and easiest way to perform and also makes it possible to introduce directly in the agriculture field. The engineered microbial system is the hope to expand their application as beneficial communities are used in agriculture, and thus enhance crop productivity and environmental sustainability. Though they can be superior in performance to the natural microbial inoculants, their successful existence in the soil matters [93, 94]. There is an ongoing controversy in the application of genetically engineered microbial inoculants in the field. The main drawbacks of using microbial inoculants include the narrow persistence of individual genotypes of microbes in the field, gene transfer in the soil harm between strains, uncertainty on the survivability, efficacy of the strains, and environmental threats associated with the genetically altered organism such as increased pathogenicity, emergence of pests or weeds [95]. One of the important questions considered upon introducing microbial inoculants in the field is, what happens to local soil microbial communities when alien species are introduced? Also, genetically modified/engineered microorganisms need constant monitoring of their behavior, which are very expensive and are vulnerable to biosafety restrictions. Moreover, the statutory exclusions in many countries control the extensive release of genetically altered microbes to the field because the release of genetically modified organisms may risk the environment by creating new pathogens, harm other soil microbes, and disrupt biotic communities. Similarly, role of invasive species and their deleterious effects in the soil ecosystem have received very little attention. For example, the introduction of commercial AM inoculants in the ecosystem may consider to harm the endemic AMF communities in the soil and it may seriously affect the ecosystem functioning [94].

CONCLUSION

Overall, various research methods are used these days to explore if the rhizosphere zone could be engineered to promote the growth of helpful microbes while preventing the plant pathogen growth. A profound understanding of the part of plants to shape the rhizosphere microbial population structure along with connecting the rhizosphere microbial communities over genetic engineering/molecular techniques could make a remarkable impact on sustainable agriculture management. Though it is challenging, the “biased rhizosphere” concept [96] could work well if there is the opportunity of aggravating the creation of plants of specialized compounds which could be catabolized by targeted beneficial microbial inoculants. This approach will help in analyzing bacterial competitiveness and perseverance in the phytosphere. Moreover, it opens innovative prospects for future agricultural advances based on exploiting the advantageous microbial facilities to lessen the inputs of agrochemicals, and thus achieving sustainable environmental and economic goals. Therefore, microbial biotechnology coupled with genetic engineering promises advancement in the modification of plant and pathogens for minor virulence, improvement in biological control agents, better microbial agents for bioremediation, *etc.* Functional and metabolic engineering, and synthetic biology are all terms used to describe various as-

pects needed to successfully engineer microbes. Further research on the processes involved may enhance the efficiency of genetic engineering approaches and extend the use of such system to a broader range of application, it offers an exciting, proven approach for better agricultural practices and productivity.

LIST OF ABBREVIATIONS

| | | |
|---------------|---|---|
| GMOs | = | Genetically Modified Organisms |
| GEMS | = | Genetically Engineered Microbial Systems |
| CRISPR | = | Clustered Regularly Interspaced Short Palindromic Repeats |
| SDGs | = | Sustainable Development Goals |
| AMF | = | Arbuscular Mycorrhizal Fungi |
| QS | = | Quorum Sensing |
| N-AHLs | = | Nacylhomoserine Lactones |
| PGPR | = | Plant Growth-promoting Rhizobacteria |
| PSHB | = | Plant Stress Homeostasis-Regulating Bacteria |
| IAA | = | Indole Acetic Acid |
| ACC deaminase | = | 1-aminocyclopropane-1-carboxylic Acid |
| ROS | = | Reactive Oxygen Species |
| OTUs | = | Operational Taxonomic Units |
| SMRT | = | Single Molecule Real-time Sequencing |
| PCR | = | Polymerase Chain Reaction |
| FISH | = | Fluorescence <i>In situ</i> Hybridization |
| HDR | = | Homology-directed Repair |
| NHEJ | = | Non-homologous End-joining |
| TALENs | = | Transcription Activator-like Effector Nucleases |
| ZFN | = | Zinc Finger Nucleases |
| RNAi | = | RNA Interference |
| QTL | = | Quantitative Trait Loci |

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We are grateful to our senior researchers, Maarja Öpik, Institute of Ecology and Earth Sciences University of Tartu, Estonia and Vijai Kumar Gupta, Estonian University of Life Sciences, Tartu, Estonia for their comments on an earlier version of the paper. Authors also acknowledge Urvashi Kuhad, Assistant Professor, Department of English, Ram Lal Anand College, University of Delhi South Campus, New Delhi, India for editing the language and grammar in the manuscript.

REFERENCES

- [1] Alori, E.T.; Babalola, O.O. microbial inoculants for improving crop quality and human health in Africa. *Front. Microbiol.*, **2018**, *9*, 2213. <http://dx.doi.org/10.3389/fmicb.2018.02213> PMID: 30283427
- [2] Amarger, N. Genetically modified bacteria in agriculture. *Biochimie*, **2002**, *84*(11), 1061-1072. [http://dx.doi.org/10.1016/S0300-9084\(02\)00035-4](http://dx.doi.org/10.1016/S0300-9084(02)00035-4) PMID: 12595134
- [3] Antoun, H.; Prévost, D. Ecology of plant growth promoting Rhizobacteria. *PGPR: Biocontrol and Biofertilization*; Siddiqui, Z.A., Ed.; Springer-Verlag: Berlin, Heidelberg, **2006**, pp. 1-38. http://dx.doi.org/10.1007/1-4020-4152-7_1
- [4] Augé, R.M.; Toler, H.D.; Saxton, A.M. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*, **2015**, *25*(1), 13-24. <http://dx.doi.org/10.1007/s00572-014-0585-4> PMID: 24831020
- [5] Babalola, O.O. Pectinase and cellulase enhance the control of *Abutilon theophrasti* by *Colletotrichum coccodes*. *Biocontrol Sci. Technol.*, **2007**, *17*(1), 53-61. <http://dx.doi.org/10.1080/09583150600828783>
- [6] Barea, J.M. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *J. Soil Sci. Plant Nutr.*, **2015**, *15*(2). <http://dx.doi.org/10.4067/S0718-95162015005000021>
- [7] Barea, J.M.; Werner, D.; Azcón-Guilar, C.; Azcón, R. Interactions of *Arbuscular mycorrhiza* and nitrogen-fixing symbiosis in sustainable agriculture. *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*; Werner, D.; Newton, W.E., Eds.; Springer-Verlag: Berlin, Heidelberg, **2005**, Vol. 4, pp. 199-222. http://dx.doi.org/10.1007/1-4020-3544-6_10
- [8] Barret, M.; Tan, H.; Egan, F.; Morrissey, J.P.; Reen, J.; O'Gara, F. Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. *Molecular Microbial Ecology of the Rhizosphere*; de Bruijn, F.J., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, **2013**, pp. 57-68. <http://dx.doi.org/10.1002/9781118297674.ch6>
- [9] Bashan, Y.; de-Bashan, L.E. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth- A critical assessment. *Advances in Agronomy*; Elsevier, **2010**, Vol. 108, pp. 77-136.
- [10] Baslam, M.; Garmendia, I.; Goicoechea, N. Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. *J. Agric. Food Chem.*, **2011**, *59*(10), 5504-5515. <http://dx.doi.org/10.1021/jf200501c> PMID: 21504187
- [11] Belimov, A.A.; Hontzeas, N.; Safronova, V.I.; Demchinskaya, S.V.; Piluzza, G.; Bullitta, S.; Glick, B.R. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.*, **2005**, *37*(2), 241-250. <http://dx.doi.org/10.1016/j.soilbio.2004.07.033>
- [12] Benckiser, G.; Bamforth, S.S. Role of pathogens, signal recalcitrance, and organisms shifting for ecosystem recuperation. A review. *Agronomy Sust. Developm.*, **2011**, *31*(1), 205-215. <http://dx.doi.org/10.1051/agro/2010024>
- [13] Brachi, B.; Filiault, D.; Darne, P.; Mentec, M.L.; Kerdaffrec, E.; Rabanal, F.; Anastasio, A.; Box, M.; Duncan, S.; Morton, T. Plant genes influence microbial hubs that shape beneficial leaf communities. *Microbiology*, **2017**. <http://dx.doi.org/10.1101/181198>
- [14] Brandt, K.; Barrangou, R. Applications of CRISPR technologies across the food supply chain. *Annu. Rev. Food Sci. Technol.*, **2019**, *10*(1), 133-150. <http://dx.doi.org/10.1146/annurev-food-032818-121204> PMID: 30908954
- [15] Callan, N.W. Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in Sh2 sweet corn. *Plant Dis.*, **1990**, *74*(5), 368. <http://dx.doi.org/10.1094/PD-74-0368>
- [16] Cepeda, M.V. Effects of microbial inoculants on biocontrol and plant growth promotion plant pathology. Thesis for Master of Science, Ohio State University, Plant Pathology., **2012**.

- [17] Collard, B.C.Y.; Jahufer, M.Z.Z.; Brouwer, J.B.; Pang, E.C.K. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, **2005**, *142*(1-2), 169-196. <http://dx.doi.org/10.1007/s10681-005-1681-5>
- [18] Cotter, J.G.E. *Crops-Necessary? Presentation to the National Academy of Sciences' Committee on Genetically Engineered Crops: Past Experience and Future Prospects*; Washington, DC, **2014**.
- [19] Crépin, A.; Barbey, C.; Cirou, A.; Tannières, M.; Orange, N.; Feuilloley, M.; Dessaux, Y.; Burini, J-F.; Faure, D.; Latour, X. Biological control of pathogen communication in the Rhizosphere: a novel approach applied to potato soft rot due to *Pectobacterium atrosepticum*. *Plant Soil*, **2012**, *358*(1-2), 27-37. <http://dx.doi.org/10.1007/s1104-011-1030-5>
- [20] de Vries, F.T.; Griffiths, R.I.; Bailey, M.; Craig, H.; Girlanda, M.; Gweon, H.S.; Hallin, S.; Kaisermann, A.; Keith, A.M.; Kretschmar, M.; Lemanceau, P.; Lumini, E.; Mason, K.E.; Oliver, A.; Ostle, N.; Prosser, J.I.; Thion, C.; Thomson, B.; Bardgett, R.D. Soil bacterial networks are less stable under drought than fungal networks. *Nat. Commun.*, **2018**, *9*(1), 3033. <http://dx.doi.org/10.1038/s41467-018-05516-7> PMID: 30072764
- [21] de Vries, F.T.; Shade, A. Controls on soil microbial community stability under climate change. *Front. Microbiol.*, **2013**, *4*, 265. <http://dx.doi.org/10.3389/fmicb.2013.00265> PMID: 24032030
- [22] De Vries, F.T.; Wallenstein, M.D. Below-ground connections underlying above-ground food production: a framework for optimizing ecological connections in the rhizosphere. *J. Ecol.*, **2017**, *105*(4), 913-920. <http://dx.doi.org/10.1111/1365-2745.12783>
- [23] Dodd, I.C.; Ruiz-Lozano, J.M. Microbial enhancement of crop resource use efficiency. *Curr. Opin. Biotechnol.*, **2012**, *23*(2), 236-242. <http://dx.doi.org/10.1016/j.copbio.2011.09.005> PMID: 21982722
- [24] Etesami, H.; Mirseyed Hosseini, H.; Alikhani, H.A. Bacterial biosynthesis of l-aminocyclopropane-l-carboxylate (ACC) deaminase, a useful trait to elongation and endophytic colonization of the roots of rice under constant flooded conditions. *Physiol. Mol. Biol. Plants*, **2014**, *20*(4), 425-434. <http://dx.doi.org/10.1007/s12298-014-0251-5> PMID: 25320466
- [25] Gaj, T.; Gersbach, C.A.; Barbas, C.F. III ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.*, **2013**, *31*(7), 397-405. <http://dx.doi.org/10.1016/j.tibtech.2013.04.004> PMID: 23664777
- [26] Gallart, M.; Adair, K.L.; Love, J.; Meason, D.F.; Clinton, P.W.; Xue, J.; Turnbull, M.H. Host genotype and nitrogen form shape the root microbiome of *Pinus radiata*. *Microb. Ecol.*, **2018**, *75*(2), 419-433. <http://dx.doi.org/10.1007/s00248-017-1055-2> PMID: 28875273
- [27] Ganbaatar, O.; Cao, B.; Zhang, Y.; Bao, D.; Bao, W.; Wuriyanghan, H. Knockdown of *Mythimna separata* chitinase genes via bacterial expression and oral delivery of RNAi effectors. *BMC Biotechnol.*, **2017**, *17*(1), 9. <http://dx.doi.org/10.1186/s12896-017-0328-7> PMID: 28183289
- [28] García-Fraile, P.; Menéndez, E.; Rivas, R. Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioeng.*, **2015**, *2*(3), 183-205. <http://dx.doi.org/10.3934/bioeng.2015.3.183>
- [29] García-Salamanca, A.; Molina-Henares, M.A.; van Dillewijn, P.; Solano, J.; Pizarro-Tobías, P.; Roca, A.; Duque, E.; Ramos, J.L. Bacterial diversity in the rhizosphere of maize and the surrounding carbonate-rich bulk soil. *Microb. Biotechnol.*, **2013**, *6*(1), 36-44. <http://dx.doi.org/10.1111/j.1751-7915.2012.00358.x> PMID: 22883414
- [30] Goold, H.D.; Wright, P.; Hailstones, D. Emerging opportunities for synthetic biology in agriculture. *Genes (Basel)*, **2018**, *9*(7), 341. <http://dx.doi.org/10.3390/genes9070341> PMID: 29986428
- [31] Gupta, S.; Pandey, S. ACC Deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (*Phaseolus vulgaris*) plants. *Front. Microbiol.*, **2019**, *10*, 1506. <http://dx.doi.org/10.3389/fmicb.2019.01506> PMID: 31338077
- [32] Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.*, **2015**, *79*(3), 293-320. <http://dx.doi.org/10.1128/MMBR.00050-14> PMID: 26136581
- [33] Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I.; Lorito, M. Trichoderma species-opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.*, **2004**, *2*(1), 43-56. <http://dx.doi.org/10.1038/nrmicro797> PMID: 15035008
- [34] Hart, M. M.; Antunes, P. M.; Abbott, L. K. Unknown risks to soil biodiversity from commercial fungal inoculants. *Nat. Ecol. Evol.*, **2017**, *1*(4), 0115. <http://dx.doi.org/10.1038/s41559-017-0115>
- [35] He, Y.; Wu, Z.; Tu, L.; Han, Y.; Zhang, G.; Li, C. Encapsulation and characterization of slow-release microbial fertilizer from the composites of bentonite and alginate. *Appl. Clay Sci.*, **2015**, *109-110*, 68-75. <http://dx.doi.org/10.1016/j.clay.2015.02.001>
- [36] Horton, M.W.; Bodenhausen, N.; Beilsmith, K.; Meng, D.; Muegge, B.D.; Subramanian, S.; Vetter, M.M.; Vilhjálmsson, B.J.; Nordborg, M.; Gordon, J.I.; Bergelson, J. Genome-wide association study of *Arabidopsis thaliana* leaf microbial community. *Nat. Commun.*, **2014**, *5*(1), 5320. <http://dx.doi.org/10.1038/ncomms6320> PMID: 25382143
- [37] Jacoby, R.; Peukert, M.; Succurro, A.; Koprivova, A.; Kopriva, S. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. *Front. Plant Sci.*, **2017**, *8*, 1617. <http://dx.doi.org/10.3389/fpls.2017.01617> PMID: 28974956
- [38] Jambhulkar, P.P.; Sharma, P.; Yadav, R. Delivery systems for introduction of microbial inoculants in the field. *Microbial Inoculants in Sustainable Agricultural Productivity*; Singh, D.P.; Singh, H.B.; Prabha, R., Eds.; Springer India: New Delhi, **2016**, pp. 199-218. http://dx.doi.org/10.1007/978-81-322-2644-4_13
- [39] Jones, D.L.; Hinsinger, P. The Rhizosphere: complex by design. *Plant Soil*, **2008**, *312*(1-2), 1-6. <http://dx.doi.org/10.1007/s11104-008-9774-2>
- [40] Jung, S.C.; Martinez-Medina, A.; Lopez-Raez, J.A.; Pozo, M.J. Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.*, **2012**, *38*(6), 651-664. <http://dx.doi.org/10.1007/s10886-012-0134-6> PMID: 22623151
- [41] Kloepper, J.W.; Leong, J.; Teintze, M.; Schroth, M.N. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, **1980**, *286*(5776), 885-886. <http://dx.doi.org/10.1038/286885a0>
- [42] Kunin, V.; Sorek, R.; Hugenholtz, P. Evolutionary conservation of sequence and secondary structures in CRISPR repeats. *Genome Biol.*, **2007**, *8*(4), R61. <http://dx.doi.org/10.1186/gb-2007-8-4-r61> PMID: 17442114
- [43] Liu, J.; Abdelfattah, A.; Norelli, J.; Burchard, E.; Schena, L.; Drobny, S.; Wisniewski, M. Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. *Microbiome*, **2018**, *6*(1), 18. <http://dx.doi.org/10.1186/s40168-018-0403-x> PMID: 29374490
- [44] Lugtenberg, B. Life of microbes in the rhizosphere. *Principles of Plant-Microbe Interactions*; Lugtenberg, B., Ed.; Springer International Publishing: Cham, **2015**, pp. 7-15.
- [45] Marschner, P.; Rumberger, A. Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. *Biol. Fertil. Soils*, **2004**, *40*(1), 1-6. <http://dx.doi.org/10.1007/s00374-004-0736-4>
- [46] Mendes, R.; Garbeva, P.; Raaijmakers, J.M. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.*, **2013**, *37*(5), 634-663. <http://dx.doi.org/10.1111/1574-6976.12028> PMID: 23790204
- [47] Mueller, U.G.; Sachs, J.L. Engineering microbiomes to improve plant and animal health. *Trends Microbiol.*, **2015**, *23*(10), 606-617. <http://dx.doi.org/10.1016/j.tim.2015.07.009> PMID: 26422463
- [48] Muhammad, T.; Zhang, F.; Zhang, Y.; Liang, Y. RNA interference: a natural immune system of plants to counteract biotic stressors. *Cells*, **2019**, *8*(1), 38. <http://dx.doi.org/10.3390/cells8010038> PMID: 30634662
- [49] Müller, D.B.; Vogel, C.; Bai, Y.; Vorholt, J.A. The plant microbiota: systems-level insights and perspectives. *Annu. Rev. Genet.*, **2016**, *50*(1), 211-234. <http://dx.doi.org/10.1146/annurev-genet-120215-034952> PMID: 27648643

- [50] Muñoz, I.V.; Sarrocco, S.; Malfatti, L.; Baroncelli, R.; Vannacci, G. CRISPR-Cas for fungal genome editing: a new tool for the management of plant diseases. *Front. Plant Sci.*, **2019**, *10*, 135. <http://dx.doi.org/10.3389/fpls.2019.00135> PMID: 30828340
- [51] Nguyen, T.H.; Phan, T.C.; Choudhury, A.T.M.A.; Rose, M.T.; Deaker, R.J.; Kennedy, I.R. BioGro: a plant growth-promoting biofertilizer validated by 15 years' research from laboratory selection to rice farmer's fields of the Mekong Delta. *Agro-Environmental Sustainability*; Singh, J.S.; Seneviratne, G., Eds.; Springer International Publishing: Cham, **2017**, pp. 237-254. http://dx.doi.org/10.1007/978-3-319-49724-2_11
- [52] Nicolás, C.; Hermosa, R.; Rubio, B.; Mukherjee, P.K.; Monte, E. Trichoderma genes in plants for stress tolerance- status and prospects. *Plant Sci.*, **2014**, *228*, 71-78. <http://dx.doi.org/10.1016/j.plantsci.2014.03.005> PMID: 25438787
- [53] Nora, L.C.; Westmann, C.A.; Guazzaroni, M.E.; Siddaiah, C.; Gupta, V.K.; Silva-Rocha, R. Recent advances in plasmid-based tools for establishing novel microbial chassis. *Biotechnol. Adv.*, **2019**, *37*(8), 107433. <http://dx.doi.org/10.1016/j.biotechadv.2019.107433> PMID: 31437573
- [54] O'Callaghan, M. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Appl. Microbiol. Biotechnol.*, **2016**, *100*(13), 5729-5746. <http://dx.doi.org/10.1007/s00253-016-7590-9> PMID: 27188775
- [55] Öpik, M.; Vanatoa, A.; Vanatoa, E.; Moora, M.; Davison, J.; Kalwij, J.M.; Reier, U.; Zobel, M. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol.*, **2010**, *188*(1), 223-241. <http://dx.doi.org/10.1111/j.1469-8137.2010.03334.x> PMID: 20561207
- [56] Owen, D.; Williams, A.P.; Griffith, G.W.; Withers, P.J.A. Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. *Appl. Soil Ecol.*, **2015**, *86*, 41-54. <http://dx.doi.org/10.1016/j.apsoil.2014.09.012>
- [57] Pandey, P.; Irulappan, V.; Bagavathiannan, M.V.; Senthil-Kumar, M. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physiomorphological traits. *Front. Plant Sci.*, **2017**, *8*, 537. <http://dx.doi.org/10.3389/fpls.2017.00537> PMID: 28458674
- [58] Pellegrino, E.; Öpik, M.; Bonari, E.; Ercoli, L. Responses of wheat to arbuscular mycorrhizal fungi: a meta-analysis of field studies from 1975 to 2013. *Soil Biol. Biochem.*, **2015**, *84*, 210-217. <http://dx.doi.org/10.1016/j.soilbio.2015.02.020>
- [59] Pellegrino, E.; Turrini, A.; Gamper, H.A.; Cafà, G.; Bonari, E.; Young, J.P.W.; Giovannetti, M. Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. *New Phytol.*, **2012**, *194*(3), 810-822. <http://dx.doi.org/10.1111/j.1469-8137.2012.04090.x> PMID: 22380845
- [60] Purvis, B.; Mao, Y.; Robinson, D. Three pillars of sustainability: in search of conceptual origins. *Sustain. Sci.*, **2019**, *14*(3), 681-695. <http://dx.doi.org/10.1007/s11625-018-0627-5>
- [61] Qiu, Z.; Egidi, E.; Liu, H.; Kaur, S.; Singh, B.K. New frontiers in agriculture productivity: Optimised microbial inoculants and *in situ* microbiome engineering. *Biotechnol. Adv.*, **2019**, *37*(6), 107371. <http://dx.doi.org/10.1016/j.biotechadv.2019.03.010> PMID: 30890361
- [62] Rani, A.; Bhat, M.N.; Singh, B.P. Effect of potato phylloplane fungi on potato late blight pathogen *Phytophthora infestans*. *J. Mycol. Plant Pathol.*, **2007**, *37*, 413-417.
- [63] Rani, A.; Singh, R.; Kumar, P.; Shukla, G. Pros and cons of fungicides: an overview. *Int. J. Eng. Sci. Res. Technol.*, **2017**, *6*(1), 112-117.
- [64] Rasmussen, P.U.; Bennett, A.E.; Tack, A.J.M. The impact of elevated temperature and drought on the ecology and evolution of plant-soil microbe interactions. *J. Ecol.*, **2020**, *108*(1), 337-352. <http://dx.doi.org/10.1111/1365-2745.13292>
- [65] Rekha, P.D.; Lai, W.-A.; Arun, A.B.; Young, C.-C. Effect of free and encapsulated *Pseudomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 on plant growth under gnotobiotic conditions. *Bioresour. Technol.*, **2007**, *98*(2), 447-451. <http://dx.doi.org/10.1016/j.biortech.2006.01.009> PMID: 16516465
- [66] Remus-Emsermann, M.N.P.; Lückner, S.; Müller, D.B.; Potthoff, E.; Daims, H.; Vorholt, J.A. Spatial distribution analyses of natural phyllosphere-colonizing bacteria on *Arabidopsis thaliana* revealed by fluorescence *in situ* hybridization: bacterial distribution on *Arabidopsis* phylloplanes. *Environ. Microbiol.*, **2014**, *16*(7), 2329-2340. <http://dx.doi.org/10.1111/1462-2920.12482> PMID: 24725362
- [67] Saikia, J.; Sarma, R.K.; Dhandia, R.; Yadav, A.; Bharali, R.; Gupta, V.K.; Saikia, R. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. *Sci. Rep.*, **2018**, *8*(1), 3560. <http://dx.doi.org/10.1038/s41598-018-21921-w> PMID: 29476114
- [68] Sapkota, R.; Knorr, K.; Jørgensen, L.N.; O'Hanlon, K.A.; Nicolaisen, M. Host genotype is an important determinant of the cereal phyllosphere microbiome. *New Phytol.*, **2015**, *207*(4), 1134-1144. <http://dx.doi.org/10.1111/nph.13418> PMID: 25898906
- [69] Savka, M.A.; Dessaux, Y.; McSpadden Gardener, B.B.; Mondy, S.; Kohler, P.R.A.; de Bruijn, F.J.; Rossbach, S. The "Biased Rhizosphere" Concept and Advances in the Omics Era to Study Bacterial Competitiveness and Persistence in the Phytosphere. *Molecular Microbial Ecology of the Rhizosphere*; de Bruijn, F.J., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, **2013**, pp. 1145-1161. <http://dx.doi.org/10.1002/9781118297674.ch110>
- [70] Schaeffer, S.M.; Nakata, P.A. CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field. *Plant Sci.*, **2015**, *240*, 130-142. <http://dx.doi.org/10.1016/j.plantsci.2015.09.011> PMID: 26475194
- [71] Schwartz, M.W.; Hoeksema, J.D.; Gehring, C.A.; Johnson, N.C.; Klironomos, J.N.; Abbott, L.K.; Pringle, A. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecol. Lett.*, **2006**, *9*(5), 501-515. <http://dx.doi.org/10.1111/j.1461-0248.2006.00910.x> PMID: 16643296
- [72] Sekar, J.; Prabavathy, V.R. Novel Phl-producing genotypes of finger millet rhizosphere associated pseudomonads and assessment of their functional and genetic diversity. *FEMS Microbiol. Ecol.*, **2014**, *89*(1), 32-46. <http://dx.doi.org/10.1111/1574-6941.12354> PMID: 24819774
- [73] Shand, H. Corporate Concentration in GE Crops: What Impact on Farmers, Biodiversity and Food Security? In: *Presentation to the National Academy of Sciences' Committee on Genetically Engineered Crops: Past Experience and Future Prospects*; Washington, DC, **2014**.
- [74] Shelake, R.M.; Pramanik, D.; Kim, J.-Y. Exploration of plant-microbe interactions for sustainable agriculture in CRISPR era. *Microorganisms*, **2019**, *7*(8), 269. <http://dx.doi.org/10.3390/microorganisms7080269> PMID: 31426522
- [75] Simeonov, D.R.; Marson, A. CRISPR-Based tools in immunity. *Annu. Rev. Immunol.*, **2019**, *37*(1), 571-597. <http://dx.doi.org/10.1146/annurev-immunol-042718-041522> PMID: 30698999
- [76] Subramanian, P.; Mageswari, A.; Kim, K.; Lee, Y.; Sa, T. Psychrotolerant endophytic *Pseudomonas* sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* Mill.) by activation of their antioxidant capacity. *Mol. Plant Microbe Interact.*, **2015**, *28*(10), 1073-1081. <http://dx.doi.org/10.1094/MPMI-01-15-0021-R> PMID: 26075827
- [77] Taie, H.A.; El-Mergawi, R.; Radwan, S. *Am.-Eurasian J. Agric. Environ. Sci.*, **2008**, *4*, 207-213.
- [78] Timmusk, S.; Behers, L.; Muthoni, J.; Muraya, A.; Aronsson, A.-C. Perspectives and challenges of microbial application for crop improvement. *Front. Plant Sci.*, **2017**, *8*, 49. <http://dx.doi.org/10.3389/fpls.2017.00049> PMID: 28232839
- [79] Tiwari, S.; Lata, C.; Chauhan, P.S.; Nautiyal, C.S. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol. Biochem.*, **2016**, *99*, 108-117. <http://dx.doi.org/10.1016/j.plaphy.2015.11.001> PMID: 26744996
- [80] Trabelsi, D.; Mhamdi, R. Microbial inoculants and their impact on soil microbial communities: a review. *BioMed Res. Int.*, **2013**, *2013*, 863240. <http://dx.doi.org/10.1155/2013/863240> PMID: 23957006
- [81] Trivedi, P.; Schenk, P.M.; Wallenstein, M.D.; Singh, B.K. Tiny Microbes, Big Yields: enhancing food crop production with biological solutions. *Microb. Biotechnol.*, **2017**, *10*(5), 999-1003.

- http://dx.doi.org/10.1111/1751-7915.12804 PMID: 28840959
- [82] Tu, L.; He, Y.; Shan, C.; Wu, Z. Preparation of microencapsulated *Bacillus subtilis* SL-13 seed coating agents and their effects on the growth of cotton seedlings. *BioMed Res. Int.*, **2016**, *2016*, 3251357. <http://dx.doi.org/10.1155/2016/3251357> PMID: 26885507
- [83] Umeha, S.; Singh, K. P.; Singh, R. Microbial biotechnology and sustainable agriculture. *Biotechnology for Sustainable Agriculture*; Elsevier, **2018**, pp. 185-205. <http://dx.doi.org/10.1016/B978-0-12-812160-3.00006-4>
- [84] Vega-Avila, A.D.; Gumiere, T.; Andrade, P.A.M.; Lima-Perim, J.E.; Durrer, A.; Baigori, M.; Vazquez, F.; Andreote, F.D. Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. *Antonie van Leeuwenhoek*, **2015**, *107*(2), 575-588. <http://dx.doi.org/10.1007/s10482-014-0353-7> PMID: 25527391
- [85] Verbruggen, E.; van der Heijden, M.G.A.; Rillig, M.C.; Kiers, E.T. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytol.*, **2013**, *197*(4), 1104-1109. <http://dx.doi.org/10.1111/j.1469-8137.2012.04348.x> PMID: 23495389
- [86] Wang, W.-X.; Zhu, T.-H.; Lai, F.-X.; Fu, Q. Event-specific qualitative and quantitative detection of transgenic rice Kefeng-6 by characterization of the transgene flanking sequence. *Eur. Food Res. Technol.*, **2011**, *232*(2), 297-305. <http://dx.doi.org/10.1007/s00217-010-1389-1>
- [87] Weyens, N.; van der Lelie, D.; Taghavi, S.; Newman, L.; Vangronsveld, J. Exploiting plant-microbe partnerships to improve biomass production and remediation. *Trends Biotechnol.*, **2009**, *27*(10), 591-598. <http://dx.doi.org/10.1016/j.tibtech.2009.07.006>
- [88] Whitehead, N.A.; Barnard, A.M.L.; Slater, H.; Simpson, N.J.L.; Salmond, G.P.C. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.*, **2001**, *25*(4), 365-404. <http://dx.doi.org/10.1111/j.1574-6976.2001.tb00583.x> PMID: 11524130
- [89] Woo, S.L.; Pepe, O. Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. *Front. Plant Sci.*, **2018**, *9*, 1801. <http://dx.doi.org/10.3389/fpls.2018.01801> PMID: 30564264
- [90] Woods, T.S. Pesticide Formulations. *AGR 185 in Encyclopedia of Agrochemicals*; Wiley & Sons: New York, **2003**, pp. 1-11. <http://dx.doi.org/10.1002/047126363X.agr185>
- [91] Wright, A.V.; Nuñez, J.K.; Doudna, J.A. Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering. *Cell*, **2016**, *164*(1-2), 29-44. <http://dx.doi.org/10.1016/j.cell.2015.12.035> PMID: 26771484
- [92] Xu, X.; Qi, L.S. A CRISPR-dCas toolbox for genetic engineering and synthetic biology. *J. Mol. Biol.*, **2019**, *431*(1), 34-47. <http://dx.doi.org/10.1016/j.jmb.2018.06.037> PMID: 29958882
- [93] Yadav, S.K.; Soni, R.; Rajput, A.S. Role of microbes in organic farming for sustainable agro-ecosystem. *Microorganisms for Green Revolution*; Panpatte, D.G.; Jhala, Y.K.; Shelat, H.N.; Vyas, R.V., Eds.: Singapore, **2018**, Vol. 7, pp. 241-252. http://dx.doi.org/10.1007/978-981-10-7146-1_12
- [94] Young, C.-C.; Rekha, P.D.; Lai, W.-A.; Arun, A.B. Encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid. *Biotechnol. Bioeng.*, **2006**, *95*(1), 76-83. <http://dx.doi.org/10.1002/bit.20957> PMID: 16619210
- [95] Zahran, E.; Sauerborn, J.; Abbasher, A.A.; Ahmed, E.A.; Mohukker, R.I.; Karlovsky, P.; Mohamed, E.A.; Müller-Stöver, D. "Pesta" and alginate delivery systems of *Fusarium* Spp. for biological control of *Striga hermonthica* (Del.) Benth. under sudanese field conditions. *Biol. Control*, **2008**, *44*(2), 160-168. <http://dx.doi.org/10.1016/j.biocontrol.2007.10.025>
- [96] Zeilinger, S.; Gupta, V.K.; Dahms, T.E.; Silva, R.N.; Singh, H.B.; Upadhyay, R.S.; Gomes, E.V.; Tsui, C.K.; Nayak S, C. Friends or foes? Emerging insights from fungal interactions with plants. *FEMS Microbiol. Rev.*, **2016**, *40*(2), 182-207. <http://dx.doi.org/10.1093/femsre/fuv045> PMID: 26591004