Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production

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ABSTRACT

Plants in nature are constantly exposed to a variety of abiotic and biotic stresses which limits their growth and production. Enhancing crop yield and production to feed exponentially growing global population in a sustainable manner by reduced chemical fertilization and agrochemicals will be a big challenge. Recently, the targeted application of beneficial plant microbiome and their cocktails to counteract abiotic and biotic stress is gaining momentum and becomes an exciting frontier of research. Advances in next generation sequencing (NGS) platform, gene editing technologies, metagenomics and bioinformatics approaches allows us to unravel the entangled webs of interactions of holobionts and core microbiomes for efficiently deploying the microbiome to increase crops nutrient acquisition and resistance to abiotic and biotic stress. In this review, we focused on shaping rhizosphere microbiome of susceptible host plant from resistant plant which comprises of specific type of microbial community with multiple potential benefits and targeted CRISPR/Cas9 based strategies for the manipulation of susceptibility genes in crop plants for improving plant health. This review is significant in providing first-hand information to improve fundamental understanding of the process which helps in shaping rhizosphere microbiome.

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Introduction

To feed the growing human population of 7.6 billion to an estimated 9.5–10 billion by 2050, will be a major challenge for the scientists across the globe. Recently, crop production is facing severe threat due to various abiotic and biotic stresses as well as limited land availability. In nature, plants are exposed to trillions of microbes that colonize and occupy different chambers or compartments of the plant like rhizosphere, rhizoplane, endosphere and phyllosphere, hence considered as a secondary genome of plant [1,2]. Several studies have been conducted in the greenhouse, field and in laboratory in order to minimize input cost and to provide beneficial services to the plants (Table 1). The plants and its microbiome are therefore, reported to function as metaorganism or holobiont [3,4]. The roots of crop plants creates an interface between the plant and the soil environment, thus establishing an enormous reservoir of microbial community [5,6]. Rhizosphere is the narrow zone of the plant roots surface and is of paramount importance for providing various ecosystem services, like cycling of nutrients and uptake of carbon [7,8]. To maximize the microbiome functions, we have to understand the biochemical and molecular determinant around the roots or the rhizosphere that governs the selective microbial enrichment [9–11]. Earlier, carbohydrates were recognized as the molecular determinants in the rhizosphere, but the studies validated that amino acids act as chemical determinants present in the rhizosphere [12]. Additionally, various flavonoids and secondary plant metabolites were considered as key drivers for the successive establishment of the host specific microbial population in the rhizospheric zone [13–15]. However, it’s not clear that these microbes are interacting with some plants either in positive or in a negative way as diversity of these microbes are different in different plants. Strong published evidences, showed that these plant inhabiting microbes are potential biofertilizers and biocontrol agents and can be used for sustainable crop production

![Table 1](Image)

| S. No. | Plant/crop | Rhizosphere | Endosphere | Rhizoplane | Sequencing technique used | Dominant species | References |
|-------|------------|-------------|------------|------------|--------------------------|-----------------|-----------|
| 1.    | Para grass (Urochloa mutica) | +++ | | | 16S rRNA | Bacillus, Chloroflexi, Microcoleus Clostridium, Caldilinea | [153] |
| 2.    | Wheat plants (Triticum aestivum) | +++ | | | 16S rRNA | Actinobacteria, Clostridia, Cellulosonas, Bacillus, Gallionella, Archaeabacter, Pseudomonas, Rhizobium, Xanthomonas, Sinorhizobium, Burkholderia, Pantocea, Enterobacter, Geobacter, Stenotrophomonas, Nocardia, Mycobacterium, Microbacterium | [33] |
| 3.    | Maize (Zea mays L.) | +++ | | | 16S RNA variable gene (V4-V5) | Acidobacteria, Gemmatimonas Rhodoferax | [154] |
| 4.    | Triticum aestivum (Wheat) | +++ | | | 16S RNA | Actinobacteria, Chloroflexi | [155] |
| 5.    | Aloe vera (Aloe barbadensis) | +++ | | | 16S RNA variable gene (V3-V4) | Bacillus, Firmicutes, Actinobacteria, Bacteroidetes | [156] |
| 6.    | Rice (Oryza sativa) | +++ | | | 16S RNA gene sequencing | Geodermatophilus, Actinobacteria, Actinoplanes, Streptomyces, Kocuria | [157] |
| 7.    | Triticum aestivum (Wheat) | +++ | +++ | | 16S RNA gene sequencing | Bacillus, Actobacter, Stenotrophomonas | [158] |
| 8.    | Triticum aestivum (Wheat) | +++ | | | 16S RNA gene sequencing | Azorarcus, Balneimonas, Bradyrhizobium, Gemmatimonas, Lyssobacter, Methylobacterium, Mesorhizobium, Microvirga, Rubellimicrobium, Rhodoplanes, Skermanella | [159] |
| 9.    | Soybean (Glycine max) | +++ | | | 16S RNA gene sequencing | Bacillus, Bradyrhizobium rhizobium, Stenotrophomonas, Streptomyces | [160] |
| 10.   | Lettuce (Lactuca sativa) | +++ | | | 16S RNA gene sequencing | Alkanindiges, Sphingomonas, Burkholderia, Novosphingobium, Sphingobium | [161] |
| 11.   | Salix (Willow) | +++ | | | 16S RNA gene sequencing | Pseudomonas, Sphingomonas yanoikuyae, Staphylococcus haemolyticus, Microbacterium oleovorans, Janthinobacterium lividum, Stenotrophomonas, Micrococcus luteus, Pantoce, Sphingomonas, Delphia | [162] |
| 12.   | Arabidopsis thaliana (Thale cress) | +++ | | | 16S RNA gene sequencing | Arthrobacter, Kineosporiacae, Flavobacterium, Massilu | [163] |
| 13.   | Arabidopsis thaliana (Thale cress) | +++ | +++ | +++ | 16S RNA variable gene (V5-V6) | Acidobacteria, Planctomycetes, Proteobacteria, Actinobacteria, Bacteroidetes | [164] |
| 14.   | Pennisetum | +++ | | | BOX-PCR, 16S RNA and nifH sequences | Azospirillum brasilense, Gluconacetobacter diazotrophicus, Gluconacetobacter liquefaciens, Gluconacetobacter sacchari, Burkholderia silvatlantica, Klebsiella sp., Enterobacter cloacae and Enterobacteriae | [165] |
| 15.   | Oryza sativa (Cultivated Rice) | +++ | | | Metaproteogenomic approach | Actinobacteria, Proteobacteria | [166] |
| 16.   | Populus deltoides (Poplar) | +++ | | | 16S RNA gene sequencing | Acidobacteria, Proteobacteria | [167] |
| 17.   | Sugarcane | +++ | | | 16S RNA gene sequencing | Citrobacter, Enterobacter, Pantoce, Klebsiella, Erwinia, Brevibacillus, Staphylococcus, Curtobacterium, Pseudomonas sp. | [168] |
| 18.   | Poplar (Populus deltoides) | +++ | | | 16S RNA microarray (Phylochip) | Actinobacteria, Firmicutes, Proteobacteria | [169] |
| 19.   | Avena fatua (wild oat) | +++ | | | 16S RNA microarray (Phylochip) | Actinobacteria, Firmicutes, Proteobacteria | [170] |
Studies conducted by different researchers unravel the understanding of the mechanism of beneficial microbiome for enhancing plant health and performance under different stress conditions [1,2,4,18–22]. These studies were based on the cultivable microbial diversity, whereas the uncultivable microbes have rarely been explored and there is an urgent need to explore the potential of these unseen microbial diversity [1,23].

Recent researches proved the use of beneficial microbiome in improving the crop yield and health of plants grown under limited conditions. Although, more research are needed on individual crops growing under stressed conditions to harness full microbiome potential. Moreover, the global climate change includes unpredicted weather pattern and elevated temperature which affects the overall functioning of ecosystem and rhizosphere biology, through direct and indirect mechanism. Therefore, the diversity of microbes present near the rhizosphere zone plays a pivotal role in enhancing plant growth by facilitating the acquisition of nutrition, providing defense against pest and pathogens, and helping plant to tolerate different types of abiotic and biotic stresses. Various types of abiotic stress include drought, salinity and high temperature that causes several negative impacts such as a major economic loss in crop productivity by reducing water absorption, nutrient acquisition, disease susceptibility and disturbing hormonal balance and also by affecting photosynthetic capacity of the plant [24]. However, still these beneficial microbes are not utilized on a full scale as only about 1–5% of the microbes present on the earth are cultivable remaining 95–99% of microbes are uncultivable [23]. Understanding of plant-microbe interaction has been a foremost area of research for several years. Recently, the advancement of high-throughput sequencing and NGS approaches has provided new insight into how these microbial communities are affected by different environmental factors and the crop genotype had made an entire catalog of the pathogens associated with specific crops, [25,26]. In case of plant disease a intricate interaction between a pathogen and the host plant, and the resistance/susceptibility response can involve many components [27]. Genome editing technologies like CRISPR/Cas9 have rapidly progressed and become essential genetic tools used for developing pathogen stress tolerance in plants [28]. Many studies conducted by different scientists have shown the importance of omics approaches to find out the uncultivable microbial flora however taxonomic and functional study of plant microbial flora is limited and rarely emphasized in detail. The rationale of this review is to decipher the role of cultivable and uncultivable microbial community associated with rhizosphere for maintaining growth and development of the plant, including the concept of shaping plant microbiome for sustainable crop production. Present review also highlighted the omics approaches, strategies for engineering rhizosphere microbiome of the plant and modern advancement made for the protection of plant by using CRISPR/Cas9 technology in some model crops plant in response to diseases caused by various microbes. Schematic flow of development of strategies for analyzing plant microbiome from different compartments and use of Omics approach for understanding of cultivable and uncultivable microbiome for plant growth promotion is shown here in Fig. 1.

Evolution of holobiont: plant-microbe interactions

Plants are coevolved into the world of microbes and rely on them for nutrient acquisition and protection against various abiotic and biotic stresses. Therefore, plants are found associated with a specific group of microbes interacting with one other forming assemblage of individuals often referred to as a “holobiont”. [29,30]. For selecting or shaping the plant associated microbial community requires a highly selective pressure that acts upon different components of holobiont which put great impact on fitness of plant. However, the high density of microbes found on different tissues of plant, together with more early origin of microorganism and their fast generation time as compared to their host, suggests that the microbe-microbe interactions are very important selective force sculpting composite assemblages of microbes in different compartments like rhizosphere, phyllo- and endosphere. Therefore, understanding of these microbiobial exchanges for shaping more intricate plant-associated communities of microbes, along with their consequence for host health in a more natural environment, remains sparse. Plants secrete carbon-rich substrates with the help of their roots, those labile substrates which are likely favored by microbes that could quickly assimilate them [11,31]. There are many success stories of engineering of rhizosphere microbiome [32], wherein most of the antique lineages of plants depict a strong competence to alter the relative abundance of rhizospheric microbes [33]. The differences in the root exudate chemistry had resulted in the selection of contrasting microbiomes [10,34,35]. The microbiome have great impact upon plant health and similarly the plants can also influence the rhizosphere microbiome through a variety of mechanisms [36,37]. The perfect reason behind this hypothesis there is phenotypic and genotypic variations in plant traits that guided the specific microbiome that can enhance growth by varieties of ways.

Rhizosphere microbiome

The rhizosphere is a narrow zone present in the soil near roots which provides an interface between plant roots and soil, therefore, it harbours plethora of microbes and small soil inhabiting animals [2]. There are two different compartments in the rhizosphere: the ectorhizosphere and the endorhizosphere. However, there are more habitats that are colonized by a variety of microorganism and their activity in association with roots has been characterized by many workers [2–4]. Published research has shown that, among the total diversity associated with plants only few microbes are found to be pathogenic while most of them have positive interactions and promotes plant survival and fitness [38–42]. Endophytes were underestimated from along time but now they are gaining lots of attention because of their nitrogen fixing potential [43]. Many studies have demonstrated that endophytes are present inside the root nodules of different crop plants like Rhizobium spp., and in non-nodulating strain of endophytes like Microbacterium trichothecenolyticum, Brevibacillus choshinensis, Endobacter medicaginis, and Micromonospora spp. [44–46]. The taxonomic variations among these endophytic bacterial strains colonizing the diverse parts of plants like leaves, stem and nodules of leguminous plants have been unravelled by using metagenomics approaches [47,48]. Therefore, very limited knowledge is available about endophytic bacteria that are found associated with various agricultural crops [48,49]. The physiology of plant-associated microbial community helps the plants for amelioration of various diseases and increased stress tolerance by assortment and transportation of various nutrients [50,51]. Therefore, the composition and functioning of microbiome at different compartment should be given priority to utilize their potential.

Key mechanisms adopted by host for recruiting microbial diversity

In the rhizospheric area, rhizo-deposition appears as a fuel for an initial substrate-driven community shift, that exert the greatest influence on rhizospheric microorganisms, which connect the genotype of the host dependent fine-tuning of microbial profiles in the selection of endophyte and colonizing various parts of the
roots. On the other hand, plant microbe co-evolution might provide the basis for a plant-driven selection process, resulting in active recruitment of microbiota members or at least keystone species that provide functions to the plant host. The variety of chemicals secreted by different parts of the roots into the soil acting as chemo attractants and are known as root exudates [52,53]. The root exudates released by the plants are considered as the key drivers for the establishment of the host specific microbial community in the rhizospheric zone [54]. The importance of root exudates as belowground defense substances has been underestimated for long a time. This mixture of exudates which are released by roots rely on exterior aspects, such as height of plant, age of the plant, soil parameters, photosynthetic activity of the soil and these properties vary with species to genus level [55]. These substances referred to as water soluble substances and were recently disclosed due to the latest advancement in microscopy and molecular tools [26]. Microbial communities are actively engaged in various key processes. However, these microbes inhabiting the soil are difficult to maintain the function of soil in both natural and artificially managed agricultural ecosystem.

Roots of plant secrete variety of phytochemicals that can mediate different types of associations which includes plant-faunal, plant-plants and plant-microbe associations. In general, a plant root secretes root exudates either as diffusates by passive mechanisms or as secretions by active mechanisms. The low molecular weight organic compounds are generally secreted by the roots of the plants via a passive process, whereas, uncharged and polar molecules are transferred directly by passive diffusion. Plant roots
releases variety of clues like root exudates which magnetize diversity of PGPRs [4,56]. Roots of a plants secretes about 5–21% of carbon which is photosynthetically fixed in the form of soluble sugars, vitamins, purines, inorganic ions, organic acid, and amino acids. Similarly, some secondary metabolites and a bulk of compounds, like phytoxiderophores, nucleosides, and the polysaccharide mucilage produced by root cap cell [10,57]. The roots of several plants like maize, wheat, barrel clover and rape were displayed to carry distinct microbial communities as a ramification of root exudates assimilation [58]. Micallef et al. [59] conducted his study on the model plant Arabidopsis thaliana and confer that the plant rhizosphere shows significant variation in the bacterial diversity relative to the bulk soil. Another study conducted by Badri et al., [60] had shown the root exudates produced by the ABC transporter mutant of A. thaliana, abcg30, contains a high level of different phenolic compounds and relatively low level of sugars, which leads to the formation of a unique microbial community in the rhizosphere. Recent studies conducted by different researchers had shown that rhizosphere microbiome could be significantly affected by the variations in a genes between different plant cultivar. The diversity of microbes present in the roots of transgenic A. thaliana plant predominately affected by the secretion of exogenous glucosinolates that directs the establishment of specific microbial community [61]. Studies conducted by Badri et al., [60] and Bressan et al., [62] on the basis of denaturing gradient gel electrophoresis (DGGE) revealed that the microbes like proteobacteria and fungi were most abundantly present [68]. The study conducted by Meier et al., [63] has depicted that the identity and abundance of root-associated fungi helps in influencing root exudation in plants [64]. Thus, exploring the process that drives the selection of the microbial community will provide new opportunities for cultivators to manipulate rhizosphere microbiome of plant in order to increase its productivity [65].

**Rhizosphere engineering: a system perspective**

Certain questions need to be answered before manipulating the rhizosphere microbiome like what are the different factors required for engineering? How would it function?. We can imagine a tool that would help us to engineer the rhizosphere in order to optimize nutrient cycling rates, water holding capacity of soil, and resistance to diversity of pathogen. It is well documented that soil microbes plays a key role in soil formation, suppressing pathogen pressure, solubilization and acquisition of nutrient. Therefore, many biological tools and approaches that tend us to manipulate the microbiome would be a key to rhizosphere engineering. Therefore, our understanding to manipulate and manage the rhizosphere microbiome is very limited. The best and most effective way to manipulate the microbiome is through bioinoculation. There are many products launched into the world market formulated by consortium of beneficial microbes like PGPR and AM fungi [66,67]. Most of the bacterial species are isolated under traditional culturing conditions inside the lab that do not emulate the soil chemical environment. These bioinoculants often show most promising results under aseptic lab and greenhouse conditions. Very little evidences support the facts that, these microbes are able to compete, establish and function as they are not persistently reproducible under natural agricultural soil. Many of these inoculants are failed under agriculture field conditions because these are easily attack by many predators or faces competition by native microbes for resources. Effective bioinoculants must have potential to form associations with other nearby microbiome, thus simulating the strong structured crosslink in native rhizosphere soils. The idea behind this approach is to add beneficial diversity of microbes so that it will improve plant functions and provides overall resistance to the plant against abiotic and biotic stress [68,69] as shown in Fig. 2.

The recent advancement in synthetic biological tools and gene editing approaches offers a distinct path to engineer microbiome with specific function [70]. Therefore, how to engineer rhizosphere of the plant is through manipulating plants traits and by crop breeding that are briefly discussed here in this review. With the successful understanding of the root architecture, host specific root exudates and other plant related traits that select specific benign microbes will help us to reshape the plant for those traits into crops by using gene editing tools like CRISPR [71]. Thus, this strategy is more promising as it emulates the associations that support the selection of beneficial microbes which will help in the evolution of the holobiont. In upcoming years, we will be able to engineer the rhizosphere purposely with the increase in sophistication in engineering approaches. For successful engineering of the rhizosphere microbiome require a systemic approach. As we understand the underlying mechanism behind how to shape the associated rhizosphere, will enhance the overall sustainability and efficiency of crop production just by imitating the beneficial symbiotic associations that took place between the soils, microbes and plants. Therefore, engineering rhizosphere is a key challenge although, some of the studies showed promising results as discussed in Table 2. Therefore, here in this review, we mainly focused on three potential approaches which have been used to shape the rhizosphere of the plant and these approaches are microbiome approach, the plant approach, and the meta-organism approach (Table 2).

**Microbiome mediated strategies for shaping rhizosphere microbiome**

Many of rhizosphere engineering strategies require, the cultivating of microbes to increase the cultivability of microbes present in rhizosphere. These cultivable microbes display certain functional capacity, but it is not clear that how these microbes will behave if they are exposed towards different environmental conditions [1,2,58]. In order to know the functionality and persistence of these microbial isolates, focused investigations are required for their beneficial impacts when used as an approach for shaping the microbiome of rhizosphere [77]. Therefore, information related to the PGPR used, as a potential biofertilizers which lives in symbiotic association with their host plants should be gathered and added into a database, so these bacterial formulations can be utilized later on in the field. Some of the rhizobia species like Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, etc and some diazotrophs that are free-living like Azospirillum, Azotobacter, Herbaspirillum, Azorarcus, and Acetobacter, etc fixes atmospheric nitrogen, mycorrhiza redeem nitrogen from ammonia (NH₃) and nitrate (NO₃) [60,72]. Different groups of PSB or phosphate solubilizing bacteria, siderophore producing bacteria, and AMF increase accessibility of diverse nutrients such as iron, phosphorous, zinc, cooper, and cadmium [16]. These rhizobacteria are also recognized as potential biocontrol agents, like Bacillus, Streptomyces and Pseudomonads and produces antibiotic compounds like phenazine, DAPG, HCN, oligomycin, bacteriocines (Nisin) as well as production of antifungal compounds like phoroglucinols, phenazines, and pyoluteorin [66,73]. Additionally, the study conducted by different scientists have depicted that the inoculation of plants with consortia of PGPR, AM fungi helps to alleviate different types of abiotic and biotic stresses by producing various defense compounds [16,17,74–77]. Strategies related to engineering rhizosphere microbiome of susceptible plant by manipulating healthy microbiome of resistant plant is shown in Fig. 2. Other than these microbes, studies on role of phytohormones on plant growth must be emphasized. Phytohormones play an essential role in growth and development of plant, and are considered as a key constituent
of plant–microbe interactions [78]. A variety of phytohormones has been reported to be produced by microbial communities such as auxins or indole-3-acetic acid (IAA), gibberellins (GA) and cytokinin. Cross talk mediated by these chemicals like jasmonic acid, salicylic acid, and ethylene and their role in activating systemic acquired resistance (SAR) and induce systemic resistance (ISR) responses in plants should be analyzed. Inoculation of plants with non-pathogenic bacteria can induce resistance against a broad range of pathogenic microbes in both below and aboveground parts. This ISR mainly depends upon jasmonic acid and ethylene signalling pathway. In this way, plants are primed to react more quickly and strongly to the pathogen attack. ISR has been detected for several microbes and for their cellular derivative determinants (so-called MAMPs), such as cell envelope elements, flagella and siderophores [79–81]. Interestingly, some PGPR elicit ISR response and promotes plant growth via emissions of a volatile organic compound (VOC) [82,83]. Well-characterized ISR-inducing microbes includes several *Pseudomonas*, *Bacillus*, and *Serratia* species and *Trichoderma harzianum*.

Moreover, strigolactones and brassinosteroids are the other compounds identified as for their hormonal activity. Inoculation of seedlings of *Miscanthus* plant with a temperate grass endophyte *Herbaspirillum frisingense* (GSF30T), stimulate shoot and root growth. The transcriptome analyses revealed that there is regulation of jasmonic acid and ethylene signalling pathway indicating that the phytohormone activity promote or modulate plant growth [84,85]. A different group of bacterial endophytes were cultured from sweet potato. The cuttings were inoculated with strains of endophytic bacteria that produce auxin and indole acetic acid (IAA). Those cuttings rapidly give rise the roots than cuttings which were not inoculated. It was demonstrated that GSF30T *Herbaspirillum frisingense* also produces IAA in the culture [86], and it was concluded that the growth of seedling in wheat plants increases when inoculated with *B. subtilis* due to production of auxin. *Azospirillum* spp. is known to stimulate plant growth by producing auxin, and by fixing nitrogen. These bacterial strains can be applied in agriculture fields for sustainable agriculture production. For example, strain B510 of *Azospirillum* sp. isolated from the stems of rice which were surface-sterilized, significantly increases yield of paddy field or rice plants by re-inoculation of seedlings, however, three strains of *Pseudomonas* enhance growth and spike length of wheat plants in field as well as in laboratory condition [87]. The biocontrol activity related to these microbes has been extensively studied not only under laboratory conditions but also in field situations, leading to several commercial products. Most products are based on *Bacillus* and *Trichoderma* strains owing to seed formulation issues, although *Pseudomonas*-based products has been used commercially in recent years [88].

Endophytic bacteria inhibit pathogenic quorum sensing by the production of specific antimicrobial products, thereby also inhibiting communication, formation of biofilm and virulence, without suppressing the growth of bacteria [89]. Endophytic bacteria also capable to degrade quorum sensing molecules and suppress formation of biofilm in *P. aeruginosa* PAO1 by production of cell-free lysates [90]. Thus, bacterial endophytes provide protection against harmful pathogens which develop resistance. Although, this quo-

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**Fig. 2.** Engineering rhizosphere microbiome of susceptible plant by manipulating healthy microbiome from resistant plant.
Table 2
Advantages and disadvantages of different strategies used in shaping the rhizosphere microbiome.

| Approach                  | Methods                                             | Mechanisms                                                                 | Advantages                                                                                           | Disadvantages                                                                                     | References |
|---------------------------|-----------------------------------------------------|----------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|------------|
| Microbiome-mediated       | Use of microbial formulation (biofertilizers)       | Application of PGPR, AMF, rhizobia, endophytes and Ecto mycorrhiza          | • Enhance plant performance and biocontrol against diseases. <br>• Production of Phytohormone Increases. <br>• SAR – ISR in the plant. <br>• Improve soil fertility of the soil. <br>• Helps in nitrogen fixation and nodulation. | At the time of inoculation very high microbial density is established but it decline over time after inoculation | [2,67,171] |
|                           |                                                     | Recombinant microbial strains.                                              | • Transferring particular genes by horizontal gene transfer (HGT) which induces the expression of beneficial functions. <br>• Development of resistance resilience stability. | Undesirable & unpredictable results related to the Horizontal gene transfer. Loss of the gene of interest with time. | [92]       |
|                           |                                                     | Imposition of chemical and mechanical disturbances: antibiotics, fungicides, tillage etc. Enhanced production of exudates | • Does not need any change in infrastructure or management in the field sites. <br>• Influences the microbial diversity by enhancing the growth of some selected microbes present in the rhizosphere | No breeding program evaluates the plant lines for interactions with the soil microbiome. | [4,173]    |
|                           |                                                     | Alteration of plant resistance to disease and environmental factors.       | • Improved tolerance toward to resist adverse environmental conditions (edaphic, biological and climatic). | May produce undesirable results.                                                                 | [92,174]  |
|                           |                                                     | Mutants selection with enhanced ability to develop mutual symbiosis.       | • Improved availability of nutrient                                                                 | Produces detrimental effect under high nutrient conditions. | [175]      |
|                           |                                                     | Plants are engineered to secrete exudates that directs specific microbial diversity for providing beneficial services. | • Plant induces microbiome for beneficial functional traits like production of siderophore, anti-fungal, antimicrobial, antibiotics acts as a biocontrol agent. <br>• Improving resistance towards adverse environment conditions. Use in bioremediation of contaminants. | Genes are transferred between inter-species. After the successful engineering of the desired gene into the plant, the compounds might inactivate in the soil, and rapidly degraded, or the rate of exudation might be too slow to influence the rhizosphere. | [172,176,177] |
|                           |                                                     | Plants are engineered for producing exudates which modify properties of the soil (acidic pH, efflux of anion from the roots). | • Plant growth is enhanced at acidic or low pH, resistance salinity, alkalinity and water stress. Enhanced resistance of plant towards Al³⁺. <br>• Enhanced phosphate solubilization. Increase in shoot biomass, longer and larger root hairs. | Enzyme activities do not always lead to the accumulation of anion and enhanced efflux. The gene TaALMT1 (release of malate in the rhizosphere) needs to be activated by A³⁺. | [92,96]    |
|                           |                                                     | Generation of transgenic plants for production of quorum sensing signal molecules N-acyl-homo serine lactone (AHL). | • Blocking of communication among the members plant-associated microbial community this may lead to an increase in plant disease resistance. | Blocking communication among members of the beneficial plant associated microbial community | [172]      |
|                           |                                                     | Plants were engineered to produce an enzyme that causes degradation of the quorum sensing signals. | • Bacterial infection prevention.                                                                 | Rhizosphere populations would be able to capture and stably integrate transgenic plant DNA, in particular antibiotic resistance genes used for the selection of transgenic plants. | [178]      |

(continued on next page)
Different phenotypes like the elevation of H+-efflux from the roots in rhizosphere transformed for over expression of H+ ATP-ase protein (AVP1)-lines of Arabidopsis thaliana in order to manipulate the pH of the rhizosphere by using transgenic solani. Yang et al., [96] and Gevaudant et al., [97] had worked in populations which are antagonist against Rhizoctonia solani. Arabidopsis and Nicotiana tabacum plants, these plants were engineered to produce compounds and inoculated bacteria are engineered to degrade these compounds. Plants are engineered to produce compounds and inoculated bacteria are engineered to degrade these compounds.

Agricultural inputs

Management and selection of complementary microorganisms and plants

Crop Rotation

Plants which synthesize opine are co-inoculated with bacteria that are able to utilizing opine.

Use of mineral fertilizers like urea, sulfates, phosphates, and ammonium nitrate.

Use of organic fertilizers like composts, biosolids and animal manures.

Plants are engineered to produce compounds and inoculated bacteria are engineered to degrade these compounds. Plants which synthesize opine are co-inoculated with bacteria that are able to utilizing opine.

Use of mineral fertilizers like urea, sulfates, phosphates, and ammonium nitrate.

Use of organic fertilizers like composts, biosolids and animal manures.

In Plant-mediated strategies, plants characters of interest are manipulated by using two different approaches: genetic engineering and plant breeding. Using plant breeding techniques for selecting a specific microbial community is an interesting approach, as the main aim of this technique is to increase crop yield, by providing plant resistance towards a variety of stresses [92]. Therefore, very important taxa and functions were targeted when microbiome selection was included in plant breeding programs. For example, Neal et al., [93] in their study used the substitution of chromosome between two wheat lines for improving tolerance towards root rot disease and thereby preserving the group of beneficial bacterial populations present in rhizosphere. The study conducted by Koyama et al., [94] reported that transgenic plants have greater ability to secrete citrate from the roots which grows better on phosphate limited soil as compared to the wild type, this study suggested that crop plants with an enhanced ability to use Al-phosphate and therefore developed an enhanced ability to grow in acidic soils and tolerance towards aluminum. Therefore, the mechanism of natural soil "suppressiveness" to soil borne diseases has been unraveled. Maazzola, [95] in his study compared cultivars of wheat for their ability to suppress disease by increasing Pseudomonads populations which are antagonist against Rhizoctonia solani. Yang et al., [96] and Gevaudant et al., [97] had worked in order to manipulate the pH of the rhizosphere by using transgenic lines of Arabidopsis and Nicotiana tabacum plants, these plants were transformed for over expression of H + ATP-ase protein (AVP1) in tobacco line [98]. The study conducted by Ellouze et al., [99] in the semi-arid grasslands of North America, showed that the particular cultivars of chickpea recruit a more beneficial microbiome for shaping durum wheat plants. Many studies conducted by different researchers in order to manipulate plants by modifying production of key exudates which directs the establishment of specific plant–microbiome interaction as discussed in Table 2. However, despite of these great efforts, for developing new plant lines large-scale genetic improvement/breeding programs were given less consideration in the past. Understanding of plant microbe interaction has been a foremost area of research for several years. Current years have witnessed the surfacing of site directed alteration methods using finger nucleases (ZFNs), meganucleases, clustered repeatedly interspaced short palindrom repeat (CRISPR/Cas9) and zinc transcription activator-like effector nucleases (TALENs). Recently, CRISPR/Cas9 has largely preferred over other genome editing technologies because of its higher success rate, easy cost, easy to design, implement and more versatile [100].

**CRISPR for crop improvement**

CRISPR or clustered regularly interspaced short palindrom repeat CRISPR-associated protein 9 (Cas9) and zinc transcription activator-like effector nucleases (TALENs). Recently, CRISPR/Cas9 has largely preferred over other genome editing technologies because of its higher success rate, easy cost, easy to design, implement and more versatile [100].

**Success stories of CRISPR/Cas9: functional studies of stress-related genes**

The study conducted by Li et al., [103] successfully reported the increased plant resistant against blast disease caused by Magnaporthe oryzae by using targeted CRISPR/Cas9 mutation in ethylene responsive factor (ERF), OsERF922 in rice. The study conducted by

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**Table 2 (continued)**

| Approach               | Methods                                      | Mechanisms                                           | Advantages                                                                 | Disadvantages                                                                 | References |
|------------------------|----------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|------------|
| Meta-organism-based    | Management and selection of complementary    | Crop Rotation                                       | • Managing soil diversity by induction of suppressive soils.               | Mechanisms are not clearly understood                                       | [22]       |
| rancherismus-based      | microorganisms and plants                    |                                                      | • Improving physico-chemical characteristics of the soil.                  |                                                                               |            |
|                        |                                              |                                                      | • Elevation in organic carbon content and higher level of nutrients cycling.|                                                                               |            |
| Plants are engineered  | Plants which synthesize opine are co-        |                                                      | • Establishing a direct link between the two partners of the interaction.   |                                                                               | [113]      |
| to produce compounds   | inoculated with bacteria that are able to    |                                                      |                                                                           |                                                                               |            |
| and inoculated         | utilizing opine.                             |                                                      |                                                                           |                                                                               |            |
| bacteria are engineered|                                              |                                                      |                                                                           |                                                                               |            |
| to degrade these       |                                              |                                                      |                                                                           |                                                                               |            |
| compounds.             |                                              |                                                      |                                                                           |                                                                               |            |
| Agricultural inputs    | Use of mineral fertilizers like urea,        | • Indirectly enhances biological activity of the soil| Fertilization of N lowers pH of soil and promotes acidification in the soil and fertilization of P affect AMF root colonization. |                                                                               | [17]       |
|                        | sulfates, phosphates, and ammonium nitrate.  | and crop residue.                                   |                                                                           |                                                                               |            |
|                        | Use of organic fertilizers like composts,    | • Increases organic matter content in the soil and   |                                                                           |                                                                               |            |
|                        | biosolids and animal manures.                | biological activity (organic fertilizers).           |                                                                           |                                                                               |            |
|                        |                                              |                                                      |                                                                           |                                                                               |            |

Rum sensing do not impel selective pressure for developing antibiotic resistance but it is another anti virulence approach for cross-examining of drug-resistant bacteria [91].
Shan et al., [104] successfully established the appliance of CRISPR TaMLO knockout was used for creating resistance against powdery mildew disease (Caused by Blumeria graminis f. sp. Triticci (Btg)) in wheat by using TaMLO gene present in its protoplasts. Maize (Zea mays) seed is main source of phytic acid (>70%) which is often considered as an environmental contaminant because of its indigestive property. Study conducted by Liang et al., [105] on maize have reported the targeted gene knockout involved in the synthesis of phytic acid (ZmMRP4, ZmIPK, ZmIPK1A, and ZmPDS). The study conducted by Cai et al., [106] was the first study which effectively achieved CRISPR/Cas9- mediated genome editing in soybean (Glycine max) by using a distinct sgRNA for a transgene (bar) and six sgRNAs that targeted diverse sites of two endogenous soybean genes (GmSHR and GmFEI2) and examine the efficiency of the sgRNAs in a hairy root system. Zhou et al., [107] in their study reported the role of OsSWEET3, a disease susceptibility gene, and importance of its expression in rice for bacterial blight disease control caused by Xanthomonas oryzae pv. Oryzae. Study carried out by Fang and Tyler [108] used CRISPR to dislocate Avr4/6, the pathogenic virulence gene in Phytophthora sojae. Replacement of Homologous gene of Bar-Avr 4/6 by (NPT II) a marker gene stimulated by the Avr4/6/ by the virulence gene in recognition of the pathogen by plants containing β-gal in soybean, Rps4 and Rps6. Targeted CRISPR/ Cas9 tools were used for developing two OsSWEET3, knockout mutants that target its promoter, and lead to enhanced tolerance against bacterial blight in rice. Plant annexins plays a noteworthy role in plant improvement and provide plant defense against different types of environmental stresses. Shen et al., [109] reported the important role played by the annexin gene (OsAnn3) present in rice, under cold stress was examined in OsAnn3 CRISPR knockouts. Several essential traits like, crop yield and abiotic stress resistance are controlled by more than one gene. In different crop enhancement programs, many studies attempt to map these quantitative trait loci – QTL that controls various agronomically imperative traits. Many identified quantitative regions introgressed into selected lines in order to develop improved varieties. However, this introgression is tedious if the QTLs are linked closely and introducing non-target regions into elite line may cause harmful effects. CRISPR/Cas9 system can be a potent tool to introduce and study rare mutations in crop plants. Shen et al., [110] reported the function of grain number QTLs (Gn1a) and grain size (Gs2) in rice varieties which were investigated by using a CRISPR based-QTL editing approach. Present study reported that, the same QTL can have highly varied and opposing effects in different backgrounds. The study conducted by Kim et al., [111] reported the role of genome editing tool CRISPR/Cas9 in wheat protoplasts for two different abiotic stress-related genes, TaERF3, wheat ERF3 and TaDREB2, wheat dehydration responsive element binding protein 2. Study carried out by Cai et al., [112] CRISPR/Cas9-mediated targeted mutation of GmFT2a delays flowering time in soyabean. CRISPR mediated gene knockout of the soybean flowering time gene, GmFT2, was stably inheritable in the subsequent T2 generation, with homozygous GmFT2a mutants exhibit late flowering under both short-day and long-day conditions. Therefore, harnessing the CRISPR/ Cas9 system for genome editing and manipulation has accelerated research and expanded researchers’ ability to generate genetic models [28].

The meta-organism mediated strategies for shaping rhizosphere microbiome

The plant and microbes are interdependent on each other and the microbiome often called as secondary genome of the plant therefore, this microbiome may function as a meta-organism or holobiont [3]. This brings the “opine concept” that combines the orchestration of the host plants to secrete particular root exudates simultaneously with the inoculation of microbes that are engineered to degrade this substrate, which often results in the colonization of the rhizosphere by a specific type of microbial community. Hence, it was also noticed that the opines produced by transgenic plants leads to the selection of the host specific microbial community that can maintain themselves at very high concentrations, even after the transgenic plant is removed [113]. These approaches which utilize specific metabolic resources are highly peculiar.

The replacement of summer fallow with different pulses in cropping systems put positive impact over the growth of the cereal crop by enhancing soil nitrogen fertility and soil water retention as well as by increasing productive land area [5,23,114,115]. The study conducted by Yang et al., [115] showed that the field-grown yellow pea and chickpea leads to the selection of specific microbial communities in the rhizospheric zone that will enhance wheat (Triticum aestivum L.) production. The study conducted by Gan et al., [115] in the semiarid region of the Canadian prairies, crop production was intensified through the involvement of pulse crops, such as chickpeas (Cicer arietinum L.), field peas (Pisum sativum L.) and lentils (Lens culinaris Medik.) in the traditional cereal-based cropping systems. Berendsen et al., [116] indicate that plants can adjust their root microbiome upon pathogen infection and specifically recruit a group of disease resistance-inducing and growth-promoting beneficial microbes for improving their chance of survival. Bainard et al., [117] in their study shown that the crop rotation between wheat with chickpea, lentil and pea, leads to increase in size of the pathogenic fungal guild that is found associated with roots but the response of bacterial community associated with roots and soil function is unknown. Hamel et al., [118] in their study have shown that the high frequency cropping of different varieties of pulses enhances nitrogen content in soil nitrogen in 4-year crop rotation systems of the semiarid prairie.

The microbial diversity present inside the rhizosphere of the contaminated soil increases the diffusion and recycling of various nutrients, mineral and synthesis of vitamins, amino acids, phytohormones like auxin, cytokinin, gibberellins that enhances plant growth. These highly competitive microbial populations are selected by the host plant via a secretion of specialized signaling molecules or roots exudates like phytoalexins, salicylic acid, and flavonoids, carbon and nitrogen compounds, results in the transformation or degradation of pollutants due to increased microbial activity and plant intervention [119,120]. These microbes also helps in the uptake of contaminants and provide plant resistant towards pollutant stress [121–123].

Integration of metagenomics with other omics approaches for shaping rhizosphere microbiome

Most of the bacteria residing in the rhizosphere zone are unculturable and their qualitative analysis are not possible. Therefore, different culture independent approaches such as metagenomics, transcriptomics, proteomics and metabolomics are essential to investigate or analyze the rhizosphere microbiome (Table 3) [120,124–126]. The most challenging part in the study of the rhizosphere is its analysis. Recently, the use of metagenomics has been increased, as it help in to qualitatively and quantitatively analyze the microbial composition of bacteria and fungi in the rhizosphere [23,127] Additionally, metaproteomics and metatranscriptomics also provides deep insights into the translation and expression of genes [2,128,129]. The Recent advancement in analytical chemistry, particularly liquid chromatography–mass spectrometry (LC–MS) and gas chromatography-mass spectrometry (GC–MS) now allow us for untargeted approaches called as metabolomics
with highly enhanced qualitative as well as quantitative analysis of the chemical constitution of any part of the plant including the rhizosphere [130]. Nuclear magnetic resonance or NMR based metabolomics also is gaining lots of attention in this field as it's not only allows quantification of chemical compounds but also helps to elucidate the chemical structure of that compounds [130]. The collection of different root exudates under aseptic conditions is considered as an option to study plant 'exudome'. The metabolomics approaches can be combined with transcriptomics approach in order to elucidate the genes that are responsible for production of many signaling molecules in the rhizosphere of the plant [131].

High-throughput or next generation sequencing technology is expeditiously upgraded in speed, cost and quality. It is therefore, extensively used to analyze whole prokaryotic communities, colonizing different niches. The 16S rRNA gene sequencing technique is extensively used to expose various bacterial communities present in the natural sample and to construct phylogenetic associations among them and also act as a useful target for profiling of the microbiome associated in the rhizospheric zone by using metagenomic approaches. Alzubaidy et al., [137] in their study used metagenomics approaches to study microbiome of mangroves that were found in red sea and also used 454-pyrosequencing technology for studying the rhizosphere microbiome that was associated with A. marina. This study resulted in the first insights into the range of functions and diversity of microbes present in the soil as well as in the rhizosphere of Grey mangrove (Avicennia marina). Pascual et al., [138] utilizes both cultivable and non-cultivable strategies for exploring the bacterial community present in the rhizosphere of the Thymus zygis grown in Sierra Nevada National Park (Spain). Recently, metagenomics studies proves that a small ‘core’ microbial consortium residing in the rhizosphere together with an AM fungus and other beneficial microbes can be used as a bioinoculants as they interact synergistically and promote plant growth [33]. Bhattacharyya et al., [139] in their study, describe the whole genome metagenomic sequencing analysis of lowland rice which further depicts the dominance of some bacterial communities, namely, Planctomycetes, Proteobacteria, Firmicutes, Acidobacteria, and Actinobacteria.

In the same line more information can be gathered by the products secreted by the different parts of the plant such as low molecular weight compounds, as they are playing very important roles in survival of the plant under various abiotic and biotic stress conditions. The natural products that are secreted from the rhizosphere of the plants often functions as a semiochemicals that helps the plant interaction with other organisms like microorganisms, animals and other plants. Therefore, the knowledge about the biosynthesis and transportation of these signaling molecules is increasing rapidly. This will help to optimize the performance of the plant just by changing their exudation into the rhizosphere [140].

While in metatranscriptomics, total RNA from the environmental samples is sequenced, which reveals various metabolic pathways and active community members [141]. However, the rRNA dominance in metatranscriptomics samples allows robust analysis of the entire microbiome, without the prior need of selecting taxonomic groups that will be used for the study. This is less challenging than samples enrich with mRNA, which avoids PCR based step and can be carried out directly on multiple samples [142]. In a metatranscriptomics approach, researchers compare the rhizosphere microorganisms of three different crop plants like oat (Avena strigosa), that produces anti-fungal compound avenacins [143], pea (Pisum sativum), a widely grown nitrogen fixing leguminous crop, and wheat (Triticum aestivum), a major staple food crop of the world. In this study, the rhizosphere microbiome of the wild variety of oat was compared with that of a mutant that is deficient in avenacin sad1 [40]. Avenacin are triterpenoid saponins that provide a defense to oat from root pathogens like Gaeumannomyces graminis that is the causative agent and causes the great destruction. Additionally, the metatranscriptomic analysis has been used to profile the communities of microbes that are present in the oceans [144,145] and in the soil [146].

**Software's for bioinformatics analysis:** To organize the whole data that has been generated by using different ‘omics’ approaches, many tools like omeSOM, PRLime Plant and MetGen- MAP are available [147–149]. Metagenomic sequencing analysis generates a huge amount of data which requires further analysis to obtain significant results (Fig. 3 shows the usual flow of metagenomics analysis). There are different software's that are available for amplicon sequencing analysis and further used for 454 ribosomal pyro-tag sequences or for Sanger sequencing like Quantitative Insights into Microbial Ecology (QIME), MEGAN, mothur (https://www.mothur.org), and CARMA, are very important and are widely used software’s.

### Table 3
List of advance molecular techniques used for characterization of rhizosphere microbial communities.

| S. No. | Techniques used | Aim of the study | References |
|-------|-----------------|------------------|------------|
| 1.    | Amplicon gene sequencing of conserved marker genes, 16S rRNA | Terrestrial mangrove fern Acrostichum from Indian Sunderbans Unearthing microbial diversity of Taxus rhizosphere Rhizobacterial population of Arachis hypogaea Bacterial and fungal rhizosphere Communities in hydrocarbon-contaminated soils Rhizosphere of apple nurseries | [179] [155] [180] [125] [181] |
| 2.    | Metagenome sequencing | Gray mangroves (Avicennia marina) in the Red Sea Grassland plant community richness and soil edaphics 454 pyrosequencing to analyze rhizosphere fungal communities during soybean growth Rhizosphere of soybean | [144] [183,184,193] [167] [194] |
| 3.    | Metatranscriptome sequencing | Rhizosphere microbiome assemblage affected by plant development Root surface microbiome Phylosphere and rhizosphere of rice | [32] [185] [166] |
| 4.    | Metaproteomic profiling | Sugarcane rhizospheric Mycorrhizal tomato roots | [186] [187,188] |
| 5.    | Metabolic profiling | | |

[134,135,136] Shenton et al., [136] studied the effect of cultivated as well as wild variety of rice genotypes over bacterial population present in the rhizospheric zone by using metagenomic approaches. Alzubaidy et al., [137] in their study used metagenomics approaches to study microbiome of mangroves that were found in red sea and also used 454-pyrosequencing technology for studying the rhizosphere microbiome that was associated with A. marina. This study resulted in the first insights into the range of functions and diversity of microbes present in the soil as well as in the rhizosphere of Grey mangrove (Avicennia marina). Pascual et al., [138] utilizes both cultivable and non-cultivable strategies for exploring the bacterial community present in the rhizosphere of the Thymus zygis grown in Sierra Nevada National Park (Spain). Recently, metagenomics studies proves that a small ‘core’ microbial consortium residing in the rhizosphere together with an AM fungus and other beneficial microbes can be used as a bioinoculants as they interact synergistically and promote plant growth [33].
for metagenomic analysis [26,32,150,151]. The PICRUSt software connects the taxonomic classification from meta-profiling results with metabolic information [152]. Many metagenomics computational/statistical tools and databases have been evolved in last decades and some of them have been mentioned in Table 4.

**Table 4**

List of bioinformatics software’s for metagenomic data analysis.

| S. No. | Software | Access | Interface | Applications | Website address | Reference |
|--------|----------|--------|-----------|--------------|-----------------|-----------|
| 1 | FastQC | Web-based | Graphical interface | Annotation | http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ | [200] |
| 2 | EBI | Web-based | Web submission | To compare functional analyses of sequences | https://www.ebi.ac.uk/metagenomics | [189] |
| 3 | KEGG | Local | Graphical interface | Biological interpretation of genome sequences | http://www.kegg.jp/blastkoala/ | [190] |
| 4 | GraPhlAn | Local/ web based | Graphical interface | Produces high-quality visualizations of microbial genomes and metagenomes | http://segatalab.cibio.unito.it/tools/graphlan | [191] |
| 5 | MetaBAT | Local | Command line interface | Binning millions of contigs from thousands of samples | https://bitbucket.org/berkeleylab/metabat | [192] |
| 6 | deFUME | Web-based | Command line interface | Processing, annotation and visualization of functional metagenomics sequencing data | https://github.com/EvdH0/deFUME | [193] |
| 7 | MetagenomeSeq | Web-based | Command line interface | Analysis of differentially abundance of 16S rRNA gene in metatprofiling data. | http://bioconductor.org/packages/release/bioc/html/metagenomeSeq.html | [194] |
| 8 | IMG/M | Web-based | Graphical interface | Functional annotation, phylogenetic distribution of genes and comparative metagenomics analysis | https://img.jgi.doe.gov/cgi-bin/m/main.cgi | [195] |
| 9 | MetaPath | Web-based | Web submission | Identification of metabolic pathways differentially abundant among metagenomic samples | http://metapath.cshb.umd.edu/ | [196] |
| 10 | BioMaS | Web-based | Graphical interface | Taxonomic studies of environmental microbial communities | http://galaxy.cloud.ba.infn.it:8080 | [197] |
| 11 | QIIME | Web-based | Command line interface | Data trimming and filtering, diversity analysis, and visualization | http://qiime.org/ | [198] |
| 12 | Galaxy portal | Web-based | Graphical interface | Web repository of computational tools that can be run without informatics expertise | https://usegalaxy.org/ | [199] |
| 13 | MOTHUR | Local | Command line interface | Data trimming and filtering, diversity analysis, and visualization | http://www.mothur.org/ | [200] |
| 14 | MG-RAST | Web-based | Graphical interface | Processing, analyzing, sharing and disseminating metagenomic datasets | http://metagenomics.anl.gov/ | [201] |
| 15 | RDP | Web-based | Web submission | Data trimming and filtering, and diversity analysis | http://rdp.cme.msu.edu/ | [202] |
| 16 | MEGAN | Local | Graphical interface | Diversity analysis and visualization (needs similarity alignments as input) | http://ab.inf.uni-tuebingen.de/software/megan | [203] |

**Concluding remarks**

The major global challenge of these days is to produce more yields from crops with less use of fertilizer and agrochemical inputs on limited land. Here, we discussed about this emerging
field of rhizosphere microbial engineering which offers influential and exciting opportunities to fill these knowledge gaps and endow with possible answers. By exogenous inoculation of particular microbes or beneficial microbiome at large it is possible to alter the structure of the microbial community to increase disease resistance in plants and uptake of specific nutrients. In this regards, the development of so-called “microbiome-driven cropping systems” might result in the next revolution in agriculture, resulting in a more sustainable system for plant production. Furthermore, the application of multiomics approaches coupled with genome editing techniques like CRISPR for enhancing nutritional status, disease resistance and crop yield will result in the progress of Non-GMO or non-genetically modified crops with desired yield and will help in future for achieving zero hunger goal for continuously increasing human population. Future research studies will utilize synthetic biology approaches, to make full use of positive plant-microbiome interactions and employ a combination of both approaches to improve the productivity of major food and bioenergy crops under environmental stress conditions, at the same time, granting for an increased drawdown of atmospheric CO₂ to stabilize carbon pools in the soil.

Compliance with Ethics Requirements
This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References
[1] Kumar A, Vyas P, Malla MA, Dubey A. Taxonomic and functional annotation of termite degraded butea monosperma (Lam.) Kunze (Flame of the Forest). Open Microbiol J 2019;13:154–63. https://doi.org/10.2174/1874285801913010154.
[2] Dubey A, Kumar A, Abd Allah EF, Hashem A, Khan ML. Growing more with less: Breeding and developing drought resilient soybean to improve food security. Ecol Ind 2019;105:425–37. https://doi.org/10.1016/j.ecolind.2019.01.003.
[3] Lakshmanan V, Selvaraj G, Bais HP. Functional Soil Microbiome: Belowground Solutions to an Aboveground Problem. Plant Physiol 2014;166:670–700. https://doi.org/10.1104/pp.114.240314.
[4] Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, et al. Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 2019;28:2405–29. https://doi.org/10.1007/s10531-018-07059-5.
[5] Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd Allah EF. Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 2018;13:1123. https://doi.org/10.3389/fmicb.2018.01123.
[6] Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd Allah EF. Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. Front Immunol 2019;9:1–23. https://doi.org/10.3389/fimmu.2018.00788.
[7] Paul Verschuren. Time to grow: lactose that control plant growth during mild to moderate drought stress. Plant Cell Environ 2016;40:177–9. https://doi.org/10.1111/pce.12827.
[8] Metagenomics Allan E. Unrestricted access to microbial communities. Trends Genet 2014;5:1–2. https://doi.org/10.1016/j.tig.2014.02.007.
[9] Soni R, Kumar V, Suyal DC, Jain L, Goel R. Metagenomics of plant rhizosphere microbiome. Understanding Host-Microbiome Interactions – An Omics Approach: Omics of Host-Microbiome Association, 2017, p. 193–205. doi.org/10.5897/ajb11.3203.
[10] Dracatos PM, Haghdoust R, Singh D, Park RF. Exploring and exploiting the microbiome: getting the hologenome concept right: an eco-evolutionary framework for understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 2018;13:1123. https://doi.org/10.3389/fmicb.2018.01123.
[11] Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, et al. Getting the Hologenome Concept Right: a Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems 2016;1:e00028–e116. https://doi.org/10.1128/mSystems.00028-16.
[12] Hashem A, Abd Allah EF, Alqarawi AA, Radhakrishnan R, Kumar A. Plant defense approach of Bacillus subtilis (BERA 71) against Macrophomina phaseolina (Tassi) Goid in mung bean. J Plant Interact 2017;12:390–401. https://doi.org/10.14716/jpi.v12i2.2017.1373871.
[13] Hasshem A, Kumar A, Al-Dhabs AM, Alqarawi AA, Al-Arjani ABF, Singh G, et al. Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. Saudi J Biol Sci 2019;26:614–24. https://doi.org/10.1016/j.sjbs.2018.11.005.
[14] Ahmad F, Kumar A, Ashraf M, Akra NA. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (Brassica juncea L.). Afr J Biotechnol 2012;11:2694–703. https://doi.org/10.5897/ajb11.1203.
[15] Kumar A, Sharma S, Mishra S. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of Jatropha curcas L. J Plant Growth Regul 2010. https://doi.org/10.4442/1742-9145/2010-00129.
[16] Kumar A, Chanderman A, Makolomakwa M, Perunal K, Singh S. Microbial production of phytases for combating environmental phosphate pollution and other diverse applications. Crit Rev Environ Sci Technol 2015;1:36. https://doi.org/10.1080/10643389.2014.913362.
[17] Patadia A, Dubey A, Malla MA, Disouza J, Pawar S, Alqarawi AA, et al. Complete Genome Sequence of Lactobacillus plantrum Strain JDARSH, Isolated from Sheep Milk. Microbiol Resource Announ 2020;9(2). https://doi.org/10.1128/mra.01199-19.
[18] Kumar A, Daines JF, Gupta A, Sharma S, Gilbert JA, Ahmad P. Current developments in arbuscular mycorrhizal fungi research and its role in salinity stress alleviation: a biotechnological perspective. Crit Rev Biotechnol 2015;35:461–74. https://doi.org/10.3109/01479576.2014.909064.
[19] Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd-Allah EF. Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. Front Immunol 2019;9:1–23. https://doi.org/10.3389/fimmu.2018.00788.
[20] Paul Verschuren. Time to grow: lactose that control plant growth during mild to moderate drought stress. Plant Cell Environ 2016;40:177–9. https://doi.org/10.1111/pce.12827.
[21] Metagenomics Allan E. Unrestricted access to microbial communities. Trends Genet 2014;5:1–2. https://doi.org/10.1016/j.tig.2014.02.007.
[22] Soni R, Kumar V, Suyal DC, Jain L, Goel R. Metagenomics of plant rhizosphere microbiome. Understanding Host-Microbiome Interactions – An Omics Approach: Omics of Host-Microbiome Association, 2017, p. 193–205. doi.org/10.5897/ajb11.3203.
[23] Dracatos PM, Haghdoust R, Singh D, Park RF. Exploring and exploiting the microbiome: getting the hologenome concept right: an eco-evolutionary framework for understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 2018;13:1123. https://doi.org/10.3389/fmicb.2018.01123.
[24] Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, et al. Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems 2016;1:e00028–e116. https://doi.org/10.1128/mSystems.00028-16.
[25] Theis RR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, et al. Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems 2016;1:e00028–e116. https://doi.org/10.1128/mSystems.00028-16.
[26] Alqarawi AA, Hashem A, Kumar A, Al-Arjani ABF, Abd Allah EF, Dar BA, et al. Allelopathic effects of the aequorin gene ofPhotobacterium phosphoreum on growth and metabolism of Salsola villosa. Plants 2018;7:1263–73. https://doi.org/10.3390/plants2050143.13491177.
[27] Chaparro JM, Badri DV, Vivanco JM. Rhizosphere microbiome assembly is affected by plant development. ISME J 2014;8. https://doi.org/10.1038/ismej.2014.192.
[28] Valverde A, De Maayer P, Oberholster T, Henschel J, Louw MK, Cowan D. Specific microbial communities associate with the rhizosphere of Welwitschia mirabilis, a living fossil. PLoS ONE 2016;11. https://doi.org/10.1371/journal.pone.0153359.
