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Rhizosphere Microbiome Modulators: Contributions of Nitrogen Fixing Bacteria towards Sustainable Agriculture

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Abstract

Rhizosphere microbiomes which have been implicated to enhance plant growth and yield are modulated or influenced by a couple of environmental factors such as soil type, plant cultivar, climate change and anthropogenic activities. In particular, anthropogenic activity such as the use of nitrogen-based chemical fertilizers is associated with environmental destruction and this call for a more ecofriendly strategy to increase nitrogen level of agricultural land. This feat is attainable by harnessing nitrogen-fixing endophytic and free-living rhizobacteria. *Rhizobium*, *Pseudomonas*, *Azospirillum* and *Bacillus* have been found to have positive impacts on crops by enhancing both above and belowground biomass and could therefore play positive roles in achieving sustainable agriculture. Thus, it is needful to study these rhizosphere microbiomes with more sophisticated culture-independent technologies such as next generation sequencing (NGS) with the prospect of discovering novel bacteria with plant growth promoting traits. This review is therefore aimed at discussing factors that can modulate rhizosphere microbiomes with focus on the contributions of nitrogen fixing bacteria towards sustainable agricultural development and the techniques that can be used for their study.

Keywords: microbiomes; next generation sequencing, plant yield; rhizobacteria; rhizosphere; sustainable agriculture

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1.0 Introduction

The rhizosphere is the zone of the soil environment that is endlessly regulated by exudates, border cells and mucilages released by plant roots [1,2]. "Rhizodeposit nutrients, border cells, mucilages, and exudates produced by plants attract and serve as food for microorganisms (including bacteria, fungi, archaea, Oomycetes, and viruses) that are present in the rhizosphere [3]". It therefore means that plant's root exudates can influence the diversity of resident microorganisms and invertebrates in the rhizosphere and these organisms can as well influence the plants by releasing regulatory substance. Hence, rhizosphere organisms are considered as a well-developed external functional environment for plants [4-7] and they are regarded as plant's second genome [8]. Since plants are regarded as metaorganisms [9], understanding the actual contributions of rhizosphere microbiomes towards plant health and productivity is necessary.

Rhizosphere microbiomes can either positively or negatively influence host plant development. The rhizosphere is impacted directly by beneficial symbiotic microorganisms or disease-causing microorganisms and indirectly via organic matter decomposition and recycling. The formation of plant and rhizospheric microbial interaction is a highly organized process influenced by host plant and soil [10]. Indeed, plants' root exudates influence rhizosphere microbial community structure which further influences plants' phenotypic traits as it has been reported that different plant cultivars produce different root exudates involved in the establishment of multi-trophic interactions in the rhizosphere [4]. Recent investigations have also demonstrated that plant host and its developmental stage play a role in shaping rhizosphere microbiome structure [11-13]. Moreover, factors such as soil and host type determine the composition of both endophytic and free-living rhizosphere microorganisms [10,14-16] while

extrinsic factors like climate change and anthropogenic activities could also modulate the microbial population dynamics in a specific plant host [17].

In particular, anthropogenic activity such as the use of nitrogen-based chemical fertilizer is associated with environmental destruction [18]. This factor among other reasons has led to the reduction in land mass that can be cultivated for agricultural purpose. Yet, there is increase in food consumption as a result of the rising human population and this poses a great demand on large hectares of viable land for agriculture. This challenge can be overcome by harnessing rhizosphere microbiomes for different agronomic purposes especially as microbial inoculants. The idea behind this agricultural practice is to expressly decrease the use of chemical fertilizers which have a lot of negative impacts on the environment. Thus, the use of plant microbiomes has the potential of minimizing environmental pollution, the occurrence of disease out-break, enhancing plant development, health and productivity. These microorganisms which can be applied to agronomic seeds singly or in consortium include endophytic and free-living root microbiomes especially nitrogen-fixing bacteria that are known to fix atmospheric nitrogen (N) for plant use. Nitrogen (N) is among the most important mineral nutrients needed for plant growth and contemporary agriculture is heavily dependent on sufficient supply of N to enhance crop yields [18] and as such a number of microbial inoculants are needed to sustain crop development.

Researchers have consequently been giving attention to endophytic bacteria because of their role in increasing N input to the soil [19]. These efforts have demonstrated that root nodules of crops contain endophytic bacteria including species of *Rhizobium*, non-nodulating strain of *Endobacter medicaginis*, *Micromonospora* species, *Microbacterium trichothecenolyticumn* and *Brevibacillus choshinensis* [20,21]. Taxonomic distinctions among bacteria associated with the

stem, leaves and nodules of legumes Alfalfa plant (*Medicago sativa* L.) have been unveiled using culture independent technologies [22] nevertheless little is known about endophytic bacteria associated some agricultural crops [23].

This review is therefore aimed at discussing factors that can modulate rhizosphere microbiomes with focus on the contributions of nitrogen fixing bacteria towards sustainable agricultural development and the techniques that can be used for their study [24-28].

2.0 Modulators of rhizosphere microbiomes of agricultural crops

Various factors such as biotic and abiotic parameters modulate or influence the microbial diversity and composition in the region surrounding the root. The level to which microbial communities are influenced by biotic and abiotic factors is not totally understood. Biotic factors such as soil type, plant host genotype/cultivar, climate change and anthropogenic activity are some of the abiotic factors that determine the rhizosphere microbiota composition [4,5,8,29-31].

Several studies have revealed that soil has a great effect on the structure of microorganisms including mycorrhizal fungal communities in the rhizosphere [32-34]. The physicochemical qualities of the soil influence plant health and type of root exudate release with the consequential effect on the structure of the rhizosphere microbial community. Sequence analysis of the bacterial community of the rhizosphere of different *Arabidopsis thaliana* cultivars revealed that soil type greatly affects rhizosphere microbial diversity [14,15]. The differences and resemblances observe in several studies could best be understood by viewing the microbial structure of the rhizosphere as emanating from a cascade of events. Firstly, the soil can be viewed as a seed bank of microorganisms [35] and secondly, the physicochemical characteristics of the soil coupled with bio-geographical activities determine the microbial assembly of the soil environment.

Soil can differ in structure, organic matter, pH, texture and nutrient status. These soil properties can select specific microorganisms by creating conducive environments that favour certain types of microorganisms and regulate the availability of roots exudates affecting selection of microorganisms by plants. In particular, soil pH and availability of nutrient such as carbon have been observed to affect the diversity of crop pathogenic nematodes, bacteria, fungi and beneficial microorganisms [36-38]. In some cases, soil properties may lead to soil type-specific composition of rhizosphere microbiome [39]. This was further confirmed by Gelsomino, Keijzer-Wolters [40] who demonstrated that bacterial community structures were alike in soils of the same type and Latour, Corberand [41] found that soil type affected the abundance and composition of *Pseudomonas* species in flax and tomato rhizosphere. This suggests that soil properties and soil type can determine the type of microorganisms that colonize the rhizosphere, and that different soil type can contain different microbial species.

Plant cultivar/genotype influences the indigenous microorganisms present in the plant rhizosphere [34,42,43]. While physicochemical characteristics of the soil can influence the composition of soil microorganisms, plant root exudates are able to modify the rhizosphere environment that slowly alters the soil microorganisms to support the establishment of a rhizobiome [44]. These root exudates together with plant root immune system would further select those microorganisms that have the ability to colonize root surface (rhizoplane) and inner root tissue (endosphere). Microorganisms that colonize root tissues (endophytes) can have harmful or positive effects on plant species which eventually have feedback effect on rhizosphere microbiome [45]. Furthermore, certain metabolites liberated into the root region can elicit several responses in various soil microorganisms. In particular, flavonoids release from plants attract symbiotic microorganisms such as *Bradyrhizobium japonicum*, and disease-causing

121 microorganisms e.g. *Phytophthora sojae*. Naringenin flavonoid produced by legumes activates
122 germination of mycorrhizal spore and hyphal branching while catechin flavonoid produced by
123 *Combretum albiflorum* regulates quorum sensing [46,47]. Similarly, some defense metabolites
124 (e.g. pyrrolizidine alkaloids) can affect the rhizosphere microbial structure by enhancing the
125 growth of microorganisms that are able to break down these metabolites.

126 Recent study shows that variations between plant cultivar in a single gene could
127 significantly affect the rhizosphere microbiome. The release of a single exogenous glucosinolates
128 changed the microbial population on transgenic *Arabidopsis* roots [48] in which fungal and
129 alpha-proteobacteria were predominantly affected, as revealed by denaturing gradient gel
130 electrophoresis (DGGE). Report has also shown that the ABC transporter mutant of *Arabidopsis*,
131 abcg30, produce root exudates containing high amount of phenolic compounds and low quantity
132 of sugars, which also gave rise to unique rhizosphere microbiome [49].

133 Rhizosphere bacterial analysis using PhyloChip technique of three different cultivars of
134 potato grown at two separate sites revealed 2432 operational taxonomic units (OTUs) and 40%
135 of the OTUs abundance was site-specific [50]. However, the abundance of 9% of the OTUs was
136 cultivar-contingent in one or the other site, whereas only 4% of the OTUs had cultivar-
137 contingent abundance in both sites. These outcomes demonstrate not only the significance of the
138 soil in shaping rhizosphere microbial community structures, but also that certain microorganisms
139 have a special affinity for specific plant cultivars. Amazingly, variations in abundance on the
140 potato cultivars were observed for microorganisms belonging to the order Pseudomonales and
141 families Streptomomycetaceae and Micromonosporaceae, which have been broadly studied for
142 their capacity to control plant pathogenic microorganisms. This result further suggests that plant
143 cultivar can influence the accumulation of bacteria that protect the plant against pathogens.

Similarly, reports have revealed differences in the ability of wheat genotypes to assemble *Pseudomonas* species that produce antifungal metabolite 2, 4-diacetylphloroglucinol (DAPG), bringing about differences in disease suppressiveness [51,52]. Additionally, the quantity of antimicrobial substance produced on roots by certain biocontrol species differs between wheat genotypes [53]. Certain wheat genotypes were also found to differentially support biocontrol bacterial species indicating that there is a level of specificity in the association between plant cultivar and the microbial species in the rhizosphere soil environment [51].

It has been suggested that the modern way of plant breeding has probably not taken into cognizance traits that are important for plants to serve as hosts to mutualistic microorganisms [4]. In an attempt to know the genetic components in plants responsible for establishing symbiosis with rhizobacteria, three “quantitative trait loci” in the genome of a tomato plant involved in suppressing disease caused by *Bacillus cereus* were detected. Furthermore, research on microbial diversity in the rhizosphere of maize showed that host genetic factor influences the composition of the rhizosphere microbiome [12,43].

In addition, microorganisms in the soil are a possible factor that influences the structure of the rhizosphere microbiome. Several studies have examined microbial colonization of the rhizosphere by intentionally coating crop seeds with certain microorganisms [4]. Previous investigations revealed that bacterial community on cucumber seedlings roots are more similar to the soil bacterial community than the seed coat bacterial biofilm, indicating that bacterial flora of the seed surface have little or no effect on the rhizosphere microbial structure. This may not be true for microorganisms present within the seeds, since study of endophytic bacteria of maize seeds showed the presence of one of the groups of the endophytic bacteria in maize rhizosphere [54]. Seed endophytic bacteria introduction into the rhizosphere suggests that plants could

transfer certain microorganisms from generation to generation. Such carry-over effect on the structure of the rhizosphere microbiome has a significant effect on co-evolution of plant-microbial interactions in the environment [4].

2.1 *Other rhizosphere microbiome modulators: climate change and anthropogenic activities*

Microorganisms play important functions in all environments and so it is significant to understand how they respond to anthropogenic activity and climate change [55,56].

Climate change has different effects, ranging from local cooling to global warming, shifting vegetation zone and augmented extreme weather events and all these effects have indirect impacts on rhizosphere microbiome. Increase in carbon dioxide levels, a component that is alleged to be the key driver of climate change, could also directly affect rhizosphere interactions by changing root exudation patterns and soil food web structure and functioning [57-59]. The structure of the soil food web can play a significant role in ameliorating the impacts of extreme weather events [60]. Experimental modification of carbon dioxide, precipitation and temperature has also indicated that climate changes have significant effects on microbial composition and abundance in the soil [61,62]. Such transformations in microbial structure can similarly change the biogeochemical cycles/processes mediated by these microorganisms. Alterations in natural ecosystems caused by climate and anthropogenic changes can augment the impact of previously trivial biogeochemical processes or introduce new processes to the ecosystem [55].

An unraveled problem now is how microbial communities of the rhizosphere will respond to different facets of climate change. Rhizosphere microorganisms may have a greater ability to evolve than their host plants. In addition, as a result of their vast biodiversity, rhizosphere microorganisms may comprise of taxa that are acclimatized to warmer

environmental conditions. The dispersal of soil microorganisms may also permits the immigration of microbial species from warmer environments into relatively cooler soil environments. Whether rhizosphere microbial composition under a particular climate change is dependent on spread of microorganisms or on their genetic acclimatization is an important question that needs to be addressed so as to know whether the microbial communities of the rhizosphere can cope with changes caused by global climate change.

Since the inception of the industrial revolution, anthropogenic activities in natural ecosystem have escalated as a result of the rising human population, pollution and ecosystem degradation and these have greatly affected soil microbial community structures. For instance, anthropogenic transformation of forest to farm lands resulted in the homogenization of the indigenous soil bacterial structure [63]. Thus, during the last 4 decades, the increasing awareness of the destructive impacts of human activities on the environments has triggered the enactment of laws to checkmate human behavior towards the environment. Though, the emphasis of the positive change in human behavior has been on the preservation of animals and plants while the negative impact of anthropogenic activities on microbial community has been relegated. This is a serious issue because microorganisms are the first responders to ecosystem disturbance and can either improve or buffer ecosystem shift [64].

The level of chemical fertilizer application is also an important anthropogenic factor that modulates rhizosphere bacterial diversity of plants in the field [65]. Sequence analysis of bacterial communities of rice crops in fields amended with low and standard levels of nitrogen fertilizer showed that the rhizosphere microbiomes were strongly affected by the level of nitrogen fertilizer. The abundance of OTUs in the genera *Bradyrhizobium*, *Methylosinus*, and *Burkholderia* were higher in the rhizosphere microbiomes from the field of the low level

nitrogen fertilizer than standard level nitrogen fertilizer. On the contrary, the relative abundance of methanogenic archaea was higher in the field amended with standard level of fertilizer (SLF) than low level nitrogen fertilizer (LNF) field [66]. The genes *pmo*/*mmo* and *acdS* responsible for methane oxidation and plant interaction respectively were more abundant in rice rhizosphere microbiomes grown in LNF field. Similarly, functional genes for the metabolism of sulphur (S), iron (Fe), aromatic compounds and nitrogen (N) were significantly higher in the LNF rhizosphere microbiomes [67]. But, ^{13}C-labeled methane experiment and quantitative PCR (qPCR) analyses for *mcrA* and *pmoA* genes coding for methyl coenzyme-M reductase and methane monooxygenase respectively indicated that methane oxidation was more active in the rice roots cultivated in LNF field than in those grown in SLF field [67]. These outcomes indicate that low-nitrogen fertilizer management is a crucial factor that modulates rhizosphere microbiome community structure and these coupled with other negative impacts of nitrogen based fertilizers necessitate the need for a more ecofriendly means of enhancing nitrogen level of agricultural land. This feat can be achieved by harnessing nitrogen-fixing endophytic and free living rhizobacteria.

3.0 Plant endophytes and their ability to fix atmospheric nitrogen

Nodule formation which is a very efficient process of nitrogen uptake has been reviewed extensively [68,69]. However, not all bacterial species can initiate nodulation because nodulation of plants such as legumes requires a complicated plant-microbe interaction. Certain bacteria penetrate roots via cracks initiated by “lateral root emergence” as well as wounds caused by “movement through the soil” [70]. These bacteria enhance plant development and play roles in nitrogen fixation [71]. Though there is no direct evidence that endophytes fix nitrogen in their plant hosts, the possibility of the process is broadly accepted. For instance, mutant strain of

Gluconacetobacter diazotrophicus which lacks the capacity to fix nitrogen is not able to enhance its host plant growth as much as the wild type. The most studied endophyte that has the ability to fix nitrogen is *Pseudomonas stutzeri* A1501, which was first isolated in China. The bacterium has possibly obtained genes coding for nitrogenase and enzyme that are adapted to different environmental conditions. The bacterium was studied so as to understand how nitrogenase activity and nitrogen fixation process are regulated and it was found that addition of ammonia to culture media stops the process of nitrogen fixation by nitrogen fixing bacteria. This is because the gene responsible for the expression of nitrogenase is down-regulated in that condition. For instance, the transcription of *nif* genes which are needed by free living organisms is repressed in the presence of ammonia. "Interestingly, *P. stutzeri* can switch between denitrification, nitrification, and nitrogen fixation under anaerobic, aerobic, and micro-aerobic conditions, respectively". Transcriptomic study has also unveiled a formerly unknown gene that plays role in nitrogen fixing process termed *pnfA. PnfA* is controlled by similar sigma factors as *nifHDK* (which code for nitrogenase). The expression of *PnfA* genes is not directly affected by mutation; however the mutant strain exhibits reduced nitrogenase activity particularly in micro-aerobic environment.

Azoarcus species are nitrogen-fixing PGPR and the wild type of *Azoarcus* sp. BH72 which inhabits kallar grass roots was able to enhance the dry weight of the grass cultivated in an environment deficient in nitrogen when compared with "*nifK* mutant strain of BH72". Surprisingly, the bacterium can transform irreversibly from free living to endophytic forms and vice versa and as such it is not always feasible to re-isolate *Azoarcus* sp. BH72 endophytic colonies from roots [72].

Rhizobium sp. IRBG74 as well as *Azorhizobium caulinodans* which colonize rice roots have been isolated respectively from *Sesbania aculeata* and *Sesbania rostrata*. *Rhizobium* sp. IRBG74 has similarly been recovered from *Sesbania cannabina*, but being an endophyte, it does not have the potential to fix nitrogen since it lacks certain *nif* genes like *nifV*. *Rhizobium* sp. IRBG74 has now been re-categorized from the genus *Agrobacterium* to *Rhizobium* since it does not possess Ti plasmid, *fusA*, *rpoB* and 16S rRNA gene sequences. This bacterium possesses a sym-plasmid having *nifH* together with *nodA* genes [73] and it colonizes a wide range of *Sesbania* plants. Similarly, *Azorhizobium caulinodans* ORS57 is able to colonize rice and fix nitrogen in endophytic form, however this bacterium should be tested with other plants species for its endophytic infection and nitrogen fixing abilities so as to know if this potential is unique to this plant sp. or is a common characteristic. "In order to determine whether it is the plant that initiates the N_2-fixation in its bacterial symbiont (as regards nodulation), a common SYM pathway rice mutant should be tested for its ability to form endophytic symbiosis with ORS571 [74,75]". It is also essential to distinguish identified multitude of genes of *Azorhizobium* responsible for plant infection, tolerance to stress and nodulation in order to ascertain those involved in *Azorhizobium-Sesbania* mutualistic interaction and rhizobial-legume associations.

Herbaspirillum seropedicae is an additional endophyte of plant roots that have been studied extensively and it infects the roots of rice, sugarcane and sorghum. It enhances the growth of its host plant by fixing nitrogen even in soil deficient in nitrogen and oxygen content. [76]. *H. seropedicae* perhaps acquired its nitrogen fixing capability via "horizontal gene transfer" like other non-rhizobial strains. Like other disease-causing microorganisms of the genus *Herbaspirillum*, it is fascinating to know that *H. seropedica*e which is non-pathogenic has the entire genetic make up for type I, II, III, V, VI, and IV pili which it uses to facilitate

281 communication with its host plant [77]. The type III is now known to be involved in the "initial
282 signal communication" of *Bradyrhizobium elkani* and *Rhizobium* sp. NGR234 with their hosts
283 [78].

284 Scientists' attention on endophytes and their contributions to plant health and
285 productivity should not only be theoretical and thus, these microorganisms can probably be
286 utilized to improve nutrient adsorption and plant diversity.

287 **4.0 Rhizobiomes as plant growth enhancers**

288 Rhizobiomes (which could be regarded as rhizobacteria) are a group of bacteria found in
289 the rhizosphere that help to enhance the growth of their host plants by producing bioactive
290 compounds and growth factors. The rhizosphere has abundant microbial diversity and nutrients
291 such as carbon substrates than the bulk soil. Microorganisms in the rhizosphere can be
292 manipulated by wide range of complicated and highly monitored cell to cell interaction and by
293 exploring signaling molecules to monitor their habitat and modify their activities. Rhizobacteria
294 can affect nutrients absorption by plant roots directly or indirectly through nutrient
295 mobilization/immobilization or alteration in root structure/physiology respectively. Many
296 microorganisms excellent at oxidizing manganese in the rhizosphere could therefore alleviate the
297 toxic level of manganese in plants cultivated in oxygen deficient and saturated soils or enhance
298 the manganese deficiency level in aerated soil containing high amount of calcium carbonate [2].

299 Rhizobacteria are a major component of plant growth promoting rhizobacteria (PGPR), a
300 terminology that was coined over 30 years ago. PGPR are non-pathogenic bacteria that colonize
301 plant roots and promote plant development and health by helping the plant to absorb more
302 nutrients and control the proliferation of pathogens that would have been detrimental to the host
303 plant [2]. Besides plant growth promotion, inoculation of rhizobacteria as a biofertilizer enhances

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304 the soil structure without leaving any negative effects in the soil unlike the conventional
305 chemical fertilizers that have been reported to contaminate agricultural land upon application
306 [79,80]. For instance, nitrates from chemical fertilizer can contaminate underground water and
307 increase the risk of blue baby syndrome in new borne babies as well as stomach cancer in adults.
308 Chemical fertilizer and pesticides can also have adverse effects on other environmental
309 components such as surface water and soil fauna and flora.

310 Moreover, siderophores as well as antibiotics produced by some species of rhizobacteria
311 are pathogen-suppressing factors which could also be utilized for agricultural purpose. Both
312 factors have microbial antagonizing properties and are able to stimulate systemic resistance [81].

313 However, some microorganisms such as arbuscular mycorrhizal fungi (AMF) present in
314 the rhizosphere have been reported to have pesticidal traits [4,82]; a potential that could also help
315 to nullify the negative impacts of chemical pesticide application and indirectly improve above
316 and belowground plants' biomass.

317 In particular, studies have shown how several rhizobiomes influenced both above and
318 below-ground biomass (Table 1) [83-85]. It is therefore desirable to harness a more ecofriendly,
319 cost effective and natural biological entities such as rhizobacteria and mycorrhizal fungi with
320 soil-enriching, pesticidal and antimicrobial potentials for sustainable agricultural development
321 [4,11].

322 **Table 1.** Selected rhizobiomes and their contributions towards sustainable agriculture
323 development.

Rhizobacteria species	Contributions towards sustainable agriculture	References
Azospirillum	Enhanced grain yield by increasing dry	[86]

amazonense	matter, panicle number and nitrogen content at maturation.	
Pseudomonas aeruginosa	Enhanced the remediation capacity of broad bean plants cultivated in soil environment containing oil contaminants. It also helps to control plant pathogens.	[4,87,88]
Serratia liquefaciens	Enhanced the remediation capacity of broad bean plants cultivated in soil environment containing oil contaminants.	[2]
Bradyrhizobium spp.	Improved nodulation in leguminous plants as well as shoot and root growth. They also enhance plants resistance to drought and production of indole-3-acetic acid	[2,84,85,89]
Azospirillum spp.	Enhanced nitrogen content in *Vicia sativa.*	[90]
Rhizobium spp.	Enhanced significantly the height, pod number and length as well as seed weight in *Vigna mungo* and *Vigna radiate.*	[91]
Bacillus spp.	Help plants to develop resistance against pathogens and pest.	[4,88]
Sinorhizobium meliloti	Improved biomass diversity in black madic plant that was subjected to copper stress.	[84,92]
Rhizobium RL9	Increased lentil plant development,	[83]

	nitrogen content, seed protein content and seed produced under heavy metal stressed environment.	
Rhizobium MRPI	Promoted nodule formation, leghaemoglobin concentration, seed protein and seed harvest in pea plant.	[93]

The effects of rhizosphere microorganisms on agronomic crops are highlighted below.

5.0 The effects of rhizosphere microbiomes on sustainable agriculture and food security

The global world requires novel ideas for farming so as to be able to generate farm produce that can cater for world population of 6.9 billion. Actualizing food security which is the process of producing sufficient food and enhancing its quality to sustain the ever increasing population without undermining environmental protection is termed global green revolution [94]. Sustainable development in the area of agriculture is required to alleviate these issues. Development of farming practices (that are environmentally friendly), natural resources and energy conservative strategy that guarantee food security are the critical aims of sustainable agriculture as reported by National Research Council [10]. It is the view of scientists that the most likely approach to actualize this objective is to replace hazardous inorganic fertilizers and pesticides with ecofriendly formulations of symbiotic microorganisms (such as *Bradyrhizobium* spp.) that have the potential to improve crop growth while providing protection from biotic stresses (such as plants pathogens and pests) and abiotic stresses (climate change and environmental pollution).

Several studies on isolation, identification and application of microorganisms as an alternative method for chemical fertilizer utilization have been reported [95,96]. Enhancing the richness and abundance of soil microorganisms by this alternative method has also shown to improve plants health and yield [97-99]. In this case, the microbial cultures are mixed with chemical carriers using solid or liquid fermentation techniques. The microbial isolates are either incorporated to the plant in pure or mixed culture either via seed application, seedling dip, bio-priming or soil application. In addition to the use of individual microorganism, identifying suitable and functionally different microbiomes and their usage for improving crop productivity is another huge and fundamental task to embark on since the entire microbiome is important, as it is described as the plant host second genome, "the metaorganisms" [10].

Hence, accomplishing food security is dependent on the enhancement of plant growth and diversity including seed yield right from the field and one of the ways this feat can be attained is through inoculation of agricultural crops (such as soybean) with rhizospheric rhizobacteria (e.g. *Rhizobium* spp.) as briefly discussed below.

5.1 *Impact of Rhizobium inoculation on leguminous crops productivity*

Rhizobium species are bacteria with the potential to reduce atmospheric nitrogen to ammonia for their host use through the formation of nodules on the roots or stems of leguminous plants [90]. This group of bacteria has been greatly studied due to their significance in agriculture and environment [100,101]. The amendment of seeds with *Rhizobium* species enhances seed protein, nodules formation and nitrogen absorption. In a review reported by Mfilinge, Mtei [90], soybean (*Glycine max* L.) inoculated with *Rhizobium* significantly increased the crop growth and yield constituents such as number of branches bearing pod per plant, total number of pod per plant and seed number per pod. Amendment of *Vicia sativa* L. (vetch) with

Azospirillum in greenhouse and field experiments increased significantly nitrogen fixation activity, percentage nitrogen and nitrogen content. *Rhizobium leguminosarum* introduced into pea and lentil seeds was able to enhance pea nodulation, shoot/root diversity and yield of pea seed. Also, seedling height, nodule and shoot biomass of lentil were enhanced. There was also enhancement on nodulation of peanut treated with *Rhizobium* species while chicken pea treated with same species in greenhouse and field experiments resulted in significant increase in plant growth, root dry weight and number of nodules. Ravikumar [91] discovered that there was significant increase in the height, fresh weight, roots, nodules, leaves, shoots and pods number, pods length and seed weight of *Vigna mungo* and *Vigna radiate* inoculated with *Rhizobium* when compared to control experiments (Table 1). Height of soybean inoculated with *Rhizobium* in field experiment significantly increased and stem girth was also increased in greenhouse cum field house experiments [102]. Similarly in a study carried out by Nyoki and Ndakidemi [101], cowpea treated with rhizobial inoculants significantly increase the height of the crop when compared to the control counterparts.

Rhizobium introduction in leguminous crops is known for growth stimulation and is used as a substitute to the expensive conventional chemical fertilizers [90]. The use of suitable species as an inoculant in nitrogen depleted environments might be a better means to enhance the development and growth of legumes. Considering the relatively cheap rate of inoculation and the possible agricultural benefits, farmers are admonished to take advantage of these inoculants as bio-fertilizers on leguminous crops.

5.2 *Impact of Rhizobium inoculation on mineral nutrients absorption by leguminous crops*

The availability and absorption of mineral elements like phosphorus (P), magnesium (Mg), sulphur (S), calcium (Ca) and potassium (K) are very essential for the growth of plants

especially in Africa where various cropping systems involving leguminous crops such as soybeans are practiced. In particular, P absorption and mutualistic nitrogen fixation are important for the development and proper functioning of plants. The uptake of these mineral nutrients relies majorly on their concentrations, activities in the soil around the root region and their replacement ability in the soil. In legumes, this challenge can be surmounted by introducing *Rhizobium* species and essential mineral nutrients into the rhizospheric soil. Besides deficiencies in major nutrients, micronutrients such as zinc (Zn), boron (B), molybdenum (Mo) and iron (Fe) are also limiting nutrients that work against legumes productivity. It was reviewed by Mfilinge, Mtei [90], that the statistical significant increase observed for K intake was linked to *Rhizobium* inoculation into Pigeon pea (*Cajanus cajan* L. Millsp) and increase in the amount of nutrient in the environment increased the chance of plants uptake. Study carried out by Makoi, Bambara [103] on the impact of *Rhizobium* strains on mineral nutrient absorption by *Phaseolus vulgaris* showed significant increase in the uptake of P, K, Mg, Ca and S in the entire plant parts. It was reported by the author that even though the concentration of P and K skyrocketed in the root region due to *Rhizobium* introduction, the increase was only significant in the greenhouse experiment and not in the field condition. *Rhizobium* inoculation enhancement of micronutrient (such as Mn, Fe, Cu, Zn, B and Mo) uptake in the shoots, roots, pods and the entire plant with the exception of Mo intake in the roots has been reported. *Rhizobium* has also been shown to cause significant increase in Ca and sodium (Na) content and the pH of the soil [104]. The uptake of Zn, Fe, Mn and Cu by cowpea was significantly different between treatments amended with *Bradyrhizobium japonicum* and phosphorus under greenhouse and field conditions [101]. *B. japonicum* caused significant increase in the intake of Zn, Fe, Cu and Mn in soybean (*Glycine max* L.) in greenhouse condition while uptake of Fe, Cu and Mn increased significantly and that

of Zn decreased under field condition [102]. There is however limited information on the roles of different rhizobial species of legumes on the bioavailability of other mineral nutrients in bean cultivars especially in Africa. Similarly, the impacts of *Rhizobium*, P and K interaction have not been studied in detail and it is therefore important to study the potential role of *Rhizobium*, phosphorus and potassium on the bioavailability of other mineral nutrients in leguminous crops such as soybean grown in each ecological zone in Africa.

5.3 *Impact of Rhizobium inoculation on chlorophyll concentration and photosynthetic activities of leguminous crops*

The crucial regulating element for plant growth is nitrogen due to its limited availability and also beans require this element more than other mineral nutrients. Nitrogen (N) is a component of most organic compounds such as proteins, growth regulators and chlorophylls. Nitrogen deficiency can lead to stunted growth, yellowing of leaves and reduced branching in beans. Nitrogen is one of the monomeric units of proteins and is greatly required for the entire enzymatic process in the cells of plants. It is also present in many vitamins and chlorophyll and plays a role in photosynthetic process [102]. In a study carried out in greenhouse and field environments, the chlorophyll content of the leaves of common beans significantly increased upon inoculation with rhizobial species. There was also significant increase in the photosynthetic activities of plant amended with rhizobial strains by 140 and 80% for greenhouse and field experiments respectively compared to control experiments [90]. It has similarly been reported that soybean amended with *Bradyrhizobium japonicum* showed increase in chlorophyll concentration and growth factors such as height of plant, leaves number on a plant, stem width, day's number to 50% flower and pod development as against the control counterparts [102]. [101] upon studying the impacts of *Bradyrhizobium japonicum* and phosphorus inoculation on

uptake of cowpea (*Vigna unguiculata* L.), discovered that chlorophyll concentration of the leaf of cowpea significantly skyrocketed for treatment inoculated with *B. japonicum* in the field. There is however little information on the impacts of K, P and *Rhizobium* inoculation and their interactions on formation of chlorophyll in *Phaseolus vulgaris* in Tanzania which is a gap that needs to be covered research wise [90].

6.0 Nexus of PGPR, Fe acquisition, plant productivity and pathogens eradication

Besides inorganic P and N, Fe is an additional mineral nutrient that plants can obtain through symbiotic interactions with soil rhizobacteria. Some PGPR are able to sequestrate the insoluble form of Fe from the soil with the aid of siderophores making it available for the host plant [105]. The sequestration or acquisition of Fe through PGPR siderophores decreases the bioavailable iron in the rhizosphere and as such affecting the growth of fungi that might be pathogenic to the plant [106,107]. In Fe deficient soil, plant is more productive in microorganism-rich soil than in soil devoid of microorganisms, buttressing the fact that PGPR help the host plants in acquiring this limited mineral nutrient [105].

7.0 New PGPR that are related to human opportunistic pathogens

Several PGPR are phylogenetically related to some of the human opportunistic microorganisms and their potential to cause disease can easily be assessed by their ability to survive at 37^{o}C [108]. The distinctions between pathogens and PGPR can be unveiled via comparative genomics. *Stenotrophomonas maltophilia* and *S. rhizophila* DSM14405T (PGPR) are genomically similar to *S. maltophilia* K279a (a human pathogen) but the former possess genes involved in the breakdown of cell walls of bacteria and plants, iron sequestration, tolerance to salinity and spermidine synthase production [108].

Many other nitrogen fixing endophytes are also closely related to some human pathogens. In particular, *Klebsiella pneumonia* Kp342 is an endophytic nitrogen-fixer of some agricultural crops and has human pathogenic close relative (strain MGH78578). Strain Kp342 is different from strain MGH78578 since it is able to fix nitrogen and lack the genes that encode "global secondary messenger cdi-GMP" known for control of virulent components and biofilm formation. "In total, 4205 proteins (putative orthologues with the average identity of 96%, based on coding sequence prediction) were shared between these two strains, and 1107 proteins were unique to the plant associated Kp342". Surprisingly, none of the projected coding sequence of Kp342 was similar to that of the already sequenced *Azoarcus* sp. BH72 (Tkacz and Poole 2015).

8.0 Nexus of rhizobia, nodule formation and SYM pathway

Some members of the Rhizobiales such as *Bradyrhizobium* species and Beta-proteobacteria are capable of forming nodules on the roots of leguminous crops where they transform atmospheric nitrogen to ammonia for plant use and gain carbon substrate from the plant in exchange [68,109]. Actinobacteria such as species of *Frankia* form nodules in interaction with plants like Casuarina and Alder. Several bacteria are free living in the environment or may exist as endophytes in plant roots, and similarly have the capacity to fix nitrogen [6]. Nodule formation in legumes was first observed approximately 100 million years back [110] long after observing mycorrhizal colonization of plant, indicating that alteration in mycorrhizal pathway gives rise to nodulation. The existence of symbiotic common pathway (SYM pathway) in microbial association with plants raises the question of whether it serves as a route for soil microbiome to gain access to plant root tissues. Oomycetes for instance use this pathway to gain access to plant and cause havoc [111]. Although, mutant strains of rice lacking SYM pathway reveal that certain endophytic microorganisms like *Rhizobium leguminosarum* are

still able to infect plant roots [74] suggesting that this is not the only pathway available for microorganisms to gain entry into plant tissues. However, plants might be able to detect certain pathogens via the SYM pathway.

10.0 Techniques use for investigation of rhizosphere microbial community structure

Researchers can gain insights into the composition and abundance of microorganisms present in the rhizosphere of plants in natural and agricultural ecosystem by using either what is broadly classified as traditional [10] or molecular [4] technologies.

10.1 Traditional techniques

Plant microbiomes are rich and abundant and they composed of pathogens, endophytes and mutualistic symbionts. In the soil environment, bacterial count could be up to 10^6-10^7 cells/cm^2; this count was obtained by culturing soil samples in nutrient media under different growth conditions (Fig.1).

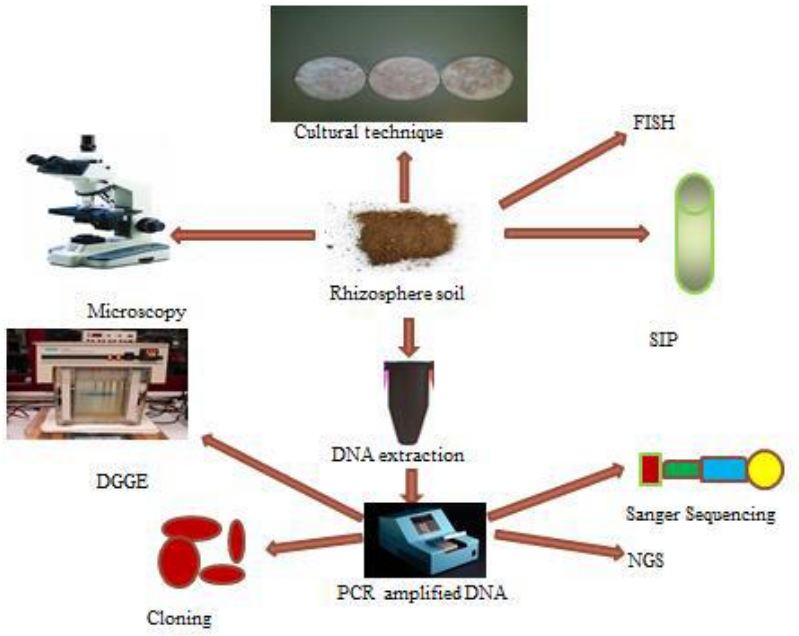

Figure 1. Schematic representation of collective techniques describing culture and un-culture based methods for the study rhizosphere microbiomes. DNA-deoxyribonucleic acid, SIP-Stable isotope probing technique, PCR-polymerase chain reaction technique, DGGE-denaturing gradient gel electrophoresis, NGS-next generation sequencing

Plants obtain different nutrients from the soil with the aid of microorganisms in the rhizosphere. These nutrients include majorly nitrogen, iron and phosphorus. These elements have the potential to influence plant growth by activating the production of plant growth regulators. Hence, the traditional techniques or culture dependent technologies are routinely used to assay for bacteria responsible for plant growth enhancement [112]. These methods involve culturing microorganisms in culture plates or broth to isolate and study plant growth promoting traits of PGPR. Some of these plant growth promoting traits include siderophores, hydrogen cyanide, phosphate solubilization and exopolysaccharide test. The traditional techniques are also used to isolate and characterize the genetic materials associated with these microorganisms and

503 unfortunately the culture based techniques are not able to capture majority of the unculturable
504 microorganisms in the rhizosphere microbiome [10] as they can only isolate approximately 1%
505 of the entire microbiome in environmental soil samples while the remaining 99% can be studied
506 via molecular techniques or culture independent technologies.

507 **10.2 Molecular techniques**

508 Attempt to profile whole microbiota commenced with the identification and application
509 of 16S RNA gene and the use of polymerase chain reaction (PCR) for characterization of
510 microorganisms. This had metamorphosed into advanced techniques such as metagenomics use
511 to explore entire microbial community. The drawbacks of these culture independent technologies
512 were recently reviewed [26,113,114] and these technologies involve metagenome sampling,
513 purification, separation, sequencing, analysis of data and interpretations. The sequencing method
514 is rapidly advancing and currently there is next generation sequencing (NGS) or high throughput
515 sequencing (HTS). The HTS technologies comprises of Roche 454 Genome Sequencer, HiSeq
516 2000, and AB SOLiDTM System [12,14,26,28].

517 Other molecular techniques involving DNA/RNA stable isotope probing (SIP) and DNA
518 arrays are also used in microbial analysis of environmental samples [26,115]. Indeed, SIP
519 technique (Fig. 1.) revealed that the root exudates emanating from maize and wheat play a role in
520 shaping microbial community of the soil surrounding the root regions [10]. The microorganisms
521 of the rhizosphere utilizing root exudates were done by analyzing only the denaturing gradient
522 gel electrophoresis (DGGE) profile of ^{13}C DNA fixed by plants amended with $^{13}CO_2$ soil while
523 microorganisms using organic matter from the soil were evaluated using ^{12}C DNA. This
524 investigation demonstrated that some classes of bacteria e.g. Sphingobacteriales and

Myxococcus could use root exudates emanating from all plants while microorganisms from the order Sphingomonadales can utilize carbon substrates of root exudates and soil organic matter.

There is presently transition from the metagenomics approaches to metatranscriptomics techniques. Metatranscriptomics give information about the diversity and functional molecules of the microbial community unlike the metagenomics that only show diversity. It was recently noted that functional diversity of the microbiomes is probably more dominant in ecological niches than genomic diversity [29,30,116]. Metatranscriptomics methods such as RNA SIP, reverse transcription quantity PCR (qPCR), cDNA analysis and pyrosequencing help to give information about the functional state of microbiomes in the rhizosphere [115]. However, culture independent technologies have a lot of challenges even though the general difficulty of qPCR and microarray techniques use for detecting gene expression of microbial community has been surmounted. The challenges entail detecting either ribosomal RNA (rRNA) or messenger RNA (mRNA), accomplishing broader analysis of an environmental RNA pool, designing effective probe and enhancing the performance of sequencing.

Metaproteomics on the other hand, deals with the analysis of protein which involves the extraction of metaproteome from environmental samples and carrying out protein fingerprinting of the extract using mass spectrometry [117,118]. Metatranscriptomics and metaproteomics which are somewhat nascent are confronted with the challenge of sampling and data procurement [117].

Recently, researchers have done little study on the rhizosphere microbial community of soybean (*Glycine max* L.) in soil ecosystem in USA [119]. Yet, much is not known about microbiomes associated with the root of most agricultural crops and more research on soil rhizospheric microbiomes of crops is therefore needed [54,105,120]. For example, no study has

548 investigated rhizosphere associated microbiota of soybean in field conditions employing HTS,
549 even though this is the 4th most produced crops in the globe [121]. Between 2015 and 2016, 320
550 million tons of soybeans were estimated to be produced.

551 Nowadays, virtually all investigations involve molecular analyses which are necessary
552 for detailed characterization of species and investigation of microbial interactions with host
553 plants in the rhizosphere. "Though there are many technical innovations in HTS that lead to
554 insightful and better understanding of the microbiome phylotypes and functions; Dini-Andreote
555 and van Elsas [114] have emphasized its hindrance on testing ecological hypotheses and the
556 current need of a 'paradigm shift' from HTS to studies on fundamental questions about yet
557 unexplored plant soil microbiota systems, especially towards phenotypic diversity of
558 rhizospheric microbiome on a spatial and temporal level"

559 **11.0 Future direction**

560 "The future trend needs to be in developing genetically modified PGPR over transgenic
561 plants for boosting plant performance, as it is simpler to modify a bacterium than complex higher
562 organisms. Moreover, instead of engineering individual crops, a single, engineered inoculant can
563 be employed for several crops, especially when using a nonspecific genus like *Azospirillum*.
564 PGPR strains development is hampered mainly by the fact that these organisms are sometimes
565 unable to survive harsh environmental conditions, including high concentrations of
566 environmental contaminants, salts, extremities of pH and temperature. Genetic
567 engineering can be used to develop PGPR strains that are effective at low inoculum doses and
568 under a variety of environmental conditions. It is urgent to develop more effective PGPR strains
569 with longer shelf lives to achieve sustainable crop production in dry land production. Recent
570 advances in the fields of microbiology, biotechnology, molecular biology and bioinformatics

have opened up the way to identify novel genes involved in drought tolerance. Concepts of micro biotechnology application in agriculture should be employed to isolate indigenous PGPR from the stress affected soils, and screening on the basis of their stress may be useful in rapid selection of efficient strains that could be used as bio-inoculants for crops grown in dry lands [122]".

12.0 Conclusions

There are myriads of microorganisms including rhizobacteria found in the ecosystem of the rhizosphere and these bacterial interactions with the plants root have been declared beneficial to sustainable agricultural development. This group of bacteria, among other merits can enhance plant development and diminish the occurrence of plant disease. New as well as uncultured microbial candidates in the rhizosphere can better be captured and studied using culture independent techniques which have the potential to analyze broad spectrum of microbial species unlike the culture based techniques. The mechanism by which these microorganisms achieve the mutual benefits on their hosts is not completely comprehended; however it has been observed that virtually all of their traits enable them to accomplish these benefits. To add to this, rhizospheric microorganisms must be competent in the sense that they should be able to strive in the rhizospheric soil that is being influenced by a several factor including soil type, plant cultivar and agricultural practices. It is advisable to match properly the suitable PGPR with the compatible host plant cum environmental condition so as to accomplish better benefits on the plant. This feat will help to reduce the usage of conventional chemical fertilizers as well as pesticides most especially if these microbial inoculants are delivered effectively to the target plant and environment.

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Igiehon NO wrote the first draft. Babalola OO provided academic input and thoroughly critiqued the article. Both authors approved the article for publication.

Competing interests

There is no competing interest between the authors

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