



*Review*

## Metagenomic study of the tomato rhizosphere soil microbiome: current state and prospect

Afeez Adesina Adedayo<sup>1</sup>, Samir Ben Romdhane<sup>2</sup> and Olubukola Oluranti Babalola<sup>1,\*</sup>

<sup>1</sup> Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa

<sup>2</sup> Laboratoire des légumineuses, Centre de Biotechnologie de Borj Cédria (CBBC), B.P. 901 Hammam-Lif, 2050 Tunisie

\* **Correspondence:** Email: [olubukola.babalola@nwu.ac.za](mailto:olubukola.babalola@nwu.ac.za); Tel.: +27-(0)-183-892-568.

**Abstract:** Plant microbiota has a variety of impacts on the plant. Some are beneficial, while some are pathogenic. This study discusses the general metagenomics procedures in processing plant-related metagenomes and focuses on the tomato plants' rhizosphere species. Metagenomics, associated with eventual DNA, is isolated from environmental samples and thus permits absolute microbial population identification. Meanwhile, the genetic content of the DNA sample obtained allows the functional capability identification and biochemical procedure of many microorganisms. This review reveals the recent utilization and application of the potential of Next-Generation Sequencing (NGS) in agriculture. It involves plant-associated microbiota, the factors driving their diversity, and plant metagenome to tackle current challenges experienced in food security. This review provides the newest methods for rapidly identifying the microbial communities inhabiting the rhizosphere soil of tomato plants.

**Keywords:** DNA extraction; microbiota; NGS; plant health; rhizobiome; tomato

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### 1. Introduction

The problem in agriculture today is how to produce abundant harvests and how the harvested crops can sustain the entire global population, which may be approximately nine billion people by

2050 [1]. The demand for food items like tomatoes will rise by 70%, according to projections by the Food and Agriculture Organization of the United Nations (FAO). The demand for fruits and vegetables is expected to double in developing nations [2]. To obtain the necessary agricultural products and increase the total harvested crops, stakeholders should improve abundant production techniques for tomatoes. To achieve the abundant agricultural yield, a mechanized agricultural farming system is required, facilitating the addition of necessary nutrients and eradicating the spoilage organisms that can affect the growth and health status of tomato plants [3].

Numerous problems have been encountered while cultivating tomatoes due to some abiotic and biotic stresses [4,5] as well as the unavailability of land due to the land tenure system [2,6]. Tomato plants interact with several microbial communities in the soil including bacteria, fungi, archaea, etc. These microbes are soil microbial communities or endosphere communities that are known as a secondary genome of the plants [7,8].

The cost of crop improvement escalated dramatically in the last ten years while inquiring about the plant microbiome was acknowledged in agricultural production [9]. Furthermore, the knowledge gained in molecular biology has improved omics techniques, substantiating the increment in the diversity of microbial communities.

Meanwhile, studying the microbiome for plants focuses on appreciating the structural and functional diversity of microorganisms related to a particular plant host in its community. The microbial plant-related communities are primarily associated with a specific gene pool interrelated with the plant host, despite microbial populations' taxonomy and functionality in the rhizosphere. The various parts of plant communities growing on its rhizosphere contain an enormous variability in microbial diversity [10]. The microorganisms dwelling in the rhizosphere of plants need nutrients and water, so they concentrate on the sources of organic matter in the soil [11]. These make the microbial population and diversity greater in rhizosphere soil found at the root region of plants. As a result of their proximity to the roots of the plants, they affect plants by improving their health status [12]. However, organic processes include phosphorus solubilization, inhibition of phytopathogens, nitrogen fixation, and soil aggregation as a result of decomposition. Soil microbes are also known to endanger plants' health if they were responsible for disease invasion on plants [13]. Therefore, tomato plants allow various routes like the production of discrete metabolites and the accumulation of specialized structures such as trichomes and hairs to endeavor and trigger the colonization of microorganisms [14].

An enormous number of microbes live in plants, including; archaea, bacteria, fungi, viruses, and oomycetes. These forms of microorganisms found in the soil are non-pathogenic but can be pathogenic depending on other conditions. The non-pathogenic organisms have more ecological relationships with the plant hosts than commensal or mutualistic associations, including competition [15]. Rhizospheric organisms include a beneficial relationship with the plant in the soil harboring them and keeping plants in place. These organisms do not harm the plant but supply nutrients and contribute to the health status of the plant by promoting plant growth and resisting disease invasion [16].

There are reasons to look into various plant species of microflora to obtain a deep perception of the microbiome's ability to improve crop productivity. Modern methods have tried to expose plant-microbe interrelation by studying the plant organs nourished with particular microorganisms [17]. Understanding the microbial community and its interrelationship with plants requires a particular observation in its surface area of research. It has several routes well expressed by scientists all around the globe [18]. Furthermore, microorganisms dwelling in the rhizosphere soil exist in diverse forms

ranging from bacteria, fungi, archaea, chlamydia, parasites, viruses, etc. They depended on plant species, selection pressure, and certain factors like improvement and other environmental characteristics [19].

The area of the soil influenced by plant roots, especially where various ecosystem procedures like the uptake of nutrients such as phosphate and nitrogen, the molecular and biochemistry nature around the rhizosphere, and the plants' roots take place, must be well known to improve the microbiome functions [20]. Adedayo et al. [21] explained carbohydrates are the molecular products of the soil around the organ of the tomato plant found in the soil. Yet, it has been reported that plant metabolites perform as a chemical determinant in the ground. Moreso, some materials like plant metabolites (carbohydrates, proteins, phenols, and flavonoids) were known as causal agents for producing some specific host microbes present at the root region in the soil [22].

Tomato (*Solanum lycopersicum*) is cultivated globally in agricultural or farming systems. This vegetable crop has been used as a distinctive crop for elementary and systematic research on fruit and vegetable quality to study the interrelationship between plants and microorganisms and other physiological characteristics [23]. On tomato-producing farms across the world, pathogens or spoilage organisms are the primary challenges troubling the production of tomatoes. Biological control mechanisms have been applied as a substitute means of controlling spoilage organisms [24]. The pathological state of tomatoes' spoilage mechanisms, including bacteria and fungi can be characterized and recognized by applying biological control agents to act against the spoilage organism's growth [25].

The rhizosphere microbial communities of tomatoes were dominated by the following listed phyla; Acidobacteria, Proteobacteria, and Bacteroidetes as reported by Cheng et al. [26]. Other phyla reported include; Actinobacteria, Firmicutes, Planctomycetes, Nitrospirae, Ascomycota, Basidiomycota, and Euryarchaeota [3]. At the genus level of classification, the following genera (*Burkholderia*, *Candidatus solibacter*, *Geodermatophilus*, *Gemmatimonas*, *Methylobacterium*, and *Rubrobacter*) have been reported to inhabit the rhizosphere of tomato plants thereby promoting the health status and improving the growth of tomato plants [3].

Metagenomics is a branch of science that reveals the study of taxonomical diversity and its functions obtained directly from environment samples based on DNA sequences [27]. According to Odelade and Babalola [28], the broad field encompasses community, ecological, and environmental genomics. It was regarded as a genomic tool by bringing together several methods. The microbial genome is applied as a role precursor for recognizing the diversity and functional genes with the help of bioinformatics to obtain detailed signification from the received data. This review portrays the current and prospective metagenomic sequencing analysis of microbial communities in the soil samples of tomato plants to enhance the production in agriculture.

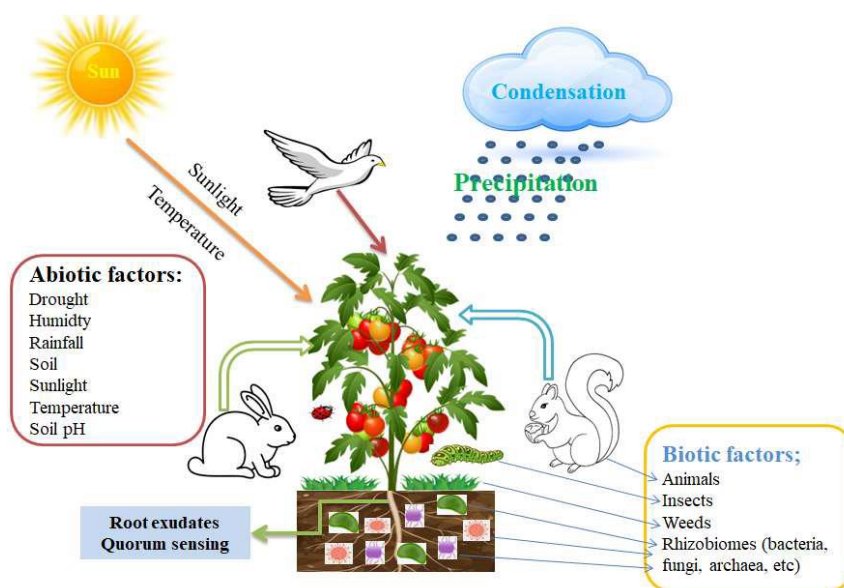
## **2. Taxonomic and functional diversity of microbial communities in the rhizosphere**

The region annotated in Figure 1 explained how the rhizosphere soil at the root region of tomato plants accommodates microbial communities and various activities regulated by the biotic and abiotic factors that include biological, chemical, and physical features. The rhizosphere soil is a region where microbial communities including bacteria, fungi, archaea, and other microbes interact with plants through their roots [29]. Diverse microorganisms embedded in the soil, structurally and functionally controlled by soil structure, types, texture, porosity, and environmental features. Backer et al. [30]

reported that root exudates and rhizosphere deposits add helpful and non-useful bacteria to the soil at the root.

The microbial communities in rhizospheric soil are more diverse due to a high concentration of nutrients or substrate utilized for bacterial, fungal, and archaeal through root exudates. Unlike soil with no plantation does not necessitate microbial community biomass in the root region. In the rhizosphere, microbial populations are involved in interactions known to affect the quality of soil and plant fitness [31]. They partake in basic activities that ascertain the stability and abundant production of agricultural and natural ecosystems. These microbial populations are advantageous to plant growth by making available the production of mineral nutrients, phytohormones, degradation and decomposition of phytotoxic compounds, and inhibition of phytopathogens [32]. Microorganisms inhabiting the rhizosphere also possess a major function in ecosystems and promote a large number of significant procedures, including carbon cycling, nitrogen cycling, nutrient acquisition, and soil formation [33]. This review reveals the significance of studying the taxonomical and functional diversity of rhizosphere microbial communities and how various components determine microbial activity in the rhizosphere soil. Nwachukwu et al. [29] supported this earlier statement, indicating that the total number of bacterial cells in a gram of rhizosphere soils ranges from  $10^8$ – $10^{12}$  due to root deposition in the rhizosphere of the crop plant. The reason for the biomass of bacteria in the rhizosphere soils was detailed in the provision of relative humidity, which adds moisture and nutrient to the soil [34].

The importance of microbial diversity in the rhizosphere soil makes them strenuous to manage. Various methods can be employed to manage soil microbes ranging from introducing advantageous microbes to reducing the activities of phytopathogens. These methods range in cost, require equipment and labor, efficient labor, and scale of application. Most times, agricultural needs are targeted by major crop management practices (crop rotation, application of manure, compost or mulch, tillage, and cover cropping) and coincidentally affect microbial communities in the soil.



**Figure 1.** Biotic and abiotic factors affecting the tomato plant.

## 2.1. Rhizosphere microbiota

In soil science, the rhizosphere is the narrow region of soil that is proportionally attributed to root exudates and associated microbiomes (bacteria, fungi, and archaea). Hence, the rhizosphere effect is the process by which biological, chemical, and physical changes in soils occur because of root secretions and rhizodeposition. The microbes present in the rhizosphere soil of the root segment of *Arabidopsis thaliana* which is not the same as those microorganisms from the bulk soil with no plantations [35]. Rani et al. [36] reported that microorganisms within the same species and distinct genotypes in the rhizosphere also produce extraordinary microbial communities.

Adeleke and Babalola [37] contributed by shedding light on types of soil, phenotypes of roots, various stages of plant growth, and the nature of the rhizosphere microbial communities. Furthermore, the plant pathogen or spoilage organisms had a specific effect on gathering microbiomes in the root where the soil had recently been established.

Certain signals produced by the plant are Quorum sensing (QS). QS was famous for signaling that counts on how the density of a rhizobacteria cell adjusts the genetic expression of microorganisms' physiological processes [38]. QS endeavors to many plant-associated bacteria to be nourished in plant-associated communities; likewise, they need QS to adjust unique phenomena, e.g., conjugation, competition in the rhizosphere, and biofilm maturation [39]. Nevertheless, QS-mimicking exudates produced from plants disturb QS, showing that plants empower microorganisms in the rhizosphere through some biological group tracts. Still, a regular shape of the conjugate deed is not apparent [40]. The community of microbial in the plant rhizosphere possesses certain necessary features. Overall, these and other factors are significant in determining the shaping of the microbiome composition, so biotic (living) and abiotic (nonliving) factors impact the community of microbes of the rhizosphere [41].

## 2.2. Soil microbiota's functionality in reducing soil-borne spoilage organisms

According to De Corato [42], suppressive soils can control spoilage organisms, thereby improving other researchers' search for various forms of disease-suppressive soil. Modern research indicates that microbiota present in some soil can produce specific environmental features for the growth of spoilage organisms, thereby producing suppressive factors controlled by the soil microbial community to spoilage organisms. However, soil microbiota could positively contribute to plant feeding for the development of the plant [43].

In disease-suppressive soils, spoilage organisms' potential subjugates to natural immunity, focusing on the following fungi: *Fusarium oxysporum*, *Phytophthora cinnamomi*, *Pythium irregular*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Pythium ultimum*, *Phytophthora capsici*, and *Phytophthora nicotianae*, and oomycetes [44]. The suppressive characteristics can be informed by adding, more importantly, antimicrobials utilized by units and microorganisms, or the process of biological control between microbiota and spoilage organisms [45].

Disease suppressions are based on biological factors made up of a collection of different actions [46]. The procedures below show that the suppressive outcome is interrelated with the natural microbiome present in the soil that deals with the soil microbiota and the plant in the community. The increase in microbial activity showcases the most necessary attributes and fungistatic [47]. These

promote soil structure and space and activate nutrient competition, eliciting microbiostasis and hyperparasitism. The production of antibiotic materials for diffusion purposes during soil organic matter decomposition leads to the release of mineral nutrients and systemic disease resistance activated in the plant host [48].

Microbial load instantly inhibiting more than one spoilage organism is one of the features where diversified procedures are activated, procuring specific protection against a broad range of spoilage organisms known as the general suppression effect [49]. In this form of suppression mechanism, antibiotics formulation, and competition are present and cannot easily be transferred from one soil to another [50]. Transfer can occur without much effort in soil characterized by little microbial community activity against few spoilage organisms. This effect is known as a specific suppression effect [51], whereby specific processes like parasitism, predation, and disease resistance activation occur. Concerning suppression, the soil classes are not commonly exclusive concerning their co-existence and suppressive activities [52].

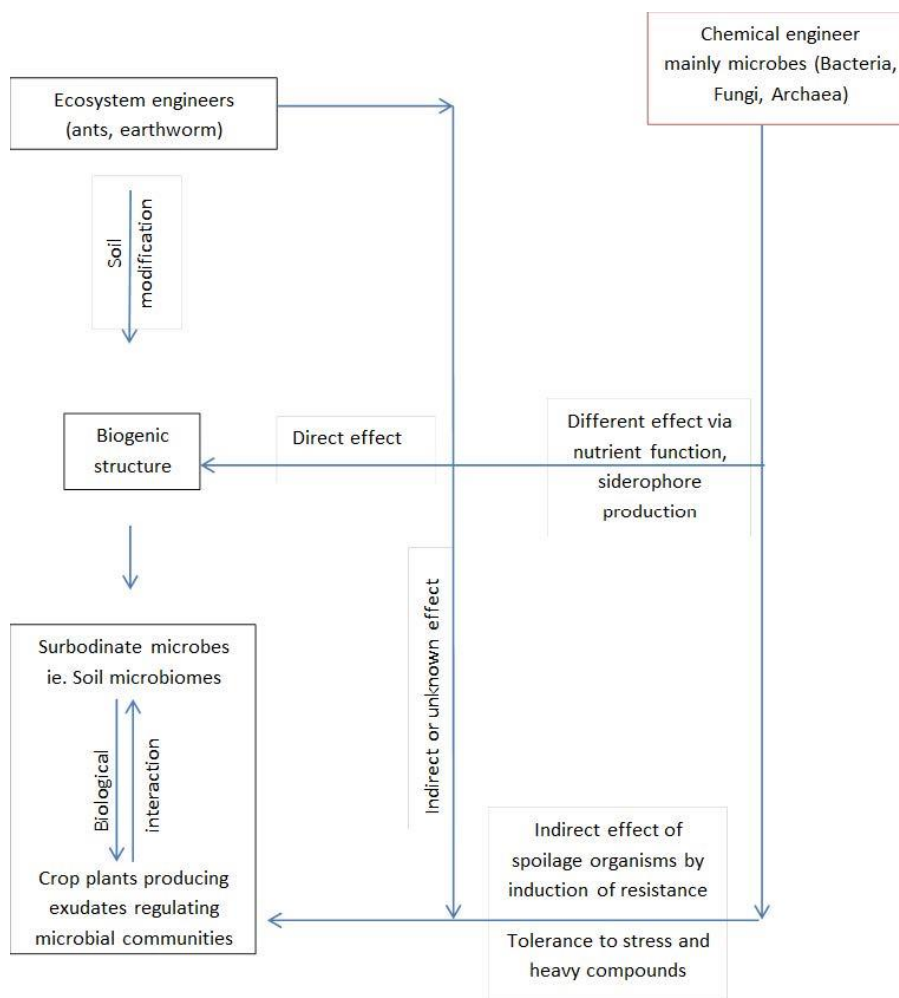
Microbiota biologically controls spoilage organisms. They include actinomycetes (*Streptomyces*), bacteria (*Bacillus*, *Paenibacillus*, and *Pseudomonas*), and filamentous fungi (*Aspergillus*, *Fusarium*, *Trichoderma*, *Gliocladium*, and *Penicillium*) that enable processes involved in conjunction with disease suppression. Specific microbiota contains Plant Growth Promoting Rhizobacteria (PGPR) of disease-suppressive compost pathogenic and non-pathogenic species like *Fusarium oxysporum* and *P. oligandrum*. They will increase plant development and vegetative propagation, enabling the host to resist or tolerate the infection [53]. Certain soils and energy impart plant growth to make required nutrients and assimilation for the production of crops by microbiota like bacteria (actinomycetes, *Bacillus*, fluorescent pseudomonads, *Paenibacillus*) and arbuscular mycorrhizal fungi (AMF), available. Fluorescent pseudomonads inhibit the spoilage organisms in the rhizosphere [54], but Adedayo et al. [21] discussed the assimilation of nutrient improvement and production of materials by plant growth-promoting substances.

Nitrogen could fix into the crop plant by *Paenibacillus brasilensis* and promote the production of auxin hormone [55] and cytokinin hormone [56]. According to De Corato [55], absorbing insoluble P by the plant is carried out by species of *Bacillus*, actinomycetes, and AMF. Furthermore, the species of *P. oligandrum* and Arbuscular Mycorrhizal Fungi can generate physical and biological changes in the plant's root system, change the rhizosphere profile, and equilibrate new biomass with root loss [57].

Phosphorus (P) is an essential plant nutrient. Its deficiency results in low agricultural income globally, mainly where the average weather conditions are unfavorable (high temperature) as in the desert and sub-Saharan countries [58]. Kalayu [59] stated that P-solubilization in the soil in the tropics is in abundance because of other essential macronutrients like aluminum and iron oxides. These oxides increase P's chance of fixation in the ground, mostly in sub-Saharan Africa. The potentially assimilated P and its usefulness have become famous globally due to its scarceness in soil [58]. In contrast, chemical derivatives containing P are minute when measured in harsh climate soil, especially in the tropics. Hence, it is necessary to know the total P in the soil to determine the P required for crop plant growth, especially in sub-Saharan Africa [60].

There is sufficient evidence that soil's potential to inhibit spoilage organisms on plants basically depends on the possibility of soil improving microbial development and other biological processes as observed in Figure 2. Among essential microorganisms, including spoilage organisms, soil organic matter, and physical, and chemical parameters like major and minor nutrient content, pH, electrical

conductivity, etc., have particular interrelations and the special route to the success of disease-suppressive compost-amended soils.



**Figure 2.** Microbial functions in rhizosphere soil.

### 2.3. The function of fungi and archaea in the rhizosphere using omics

Archaeal microbiome found in plants is unculturable while applying a conventional culture-based procedure. It is revealed by the adoption of genomic phylogenetic studies but is comprehensible in the case of fungal diversity, while omics express their usefulness. The following illustrates the variety of omics: metagenomics, metabolomics, and metatranscriptomics that provide enabling knowledge of advantageous rhizospheric microbial communities, their organization, and abundance in plant proximity found in research making use of specific crops [61]. Research on the soil's microbial community has increased significantly by applying next-generation sequencing methods and high parallel sequencing output [62] with special duties. These protection duties against non-living features include plant growth promotion by biological synthesis of auxin hormone, nutrient supply, stress (osmotic and oxidative) tolerance, and the likes suddenly uncovered in archaea ascertaining the need

to continually investigate the diversity of microbes present in the plant microbiome [63]. Several kinds of metagenomics research (Figure 2) have been done on fungi and archaea, acquiring particular organisms and unveiling functions and characteristics of microbial communities in plants enhancing agricultural practice (Salk and Kennedy 2020) and its production [64].

#### 2.4. Rhizosphere studies conducted in Africa

Several studies have been conducted globally on different rhizosphere soil. In the African continent, various studies have revealed the potential of microbial communities dwelling in the rhizosphere ranging from their microbial diversity [3,65], functional diversity [66,67], and functional genes [68,69] produced by these microbiomes to improve the health status of the plants. Gashaw et al. [70] have reported how *Pseudomonas* or *Bacillus* species have displayed their potential action against tomato wilt *Ralstonia solanacearum* in the rhizosphere soil obtained from Central Ethiopia. More studies on various crops not limited to tomato plants have been done in Africa which were revealed in Table 1 below.

**Table 1.** Some studies conducted in Africa on agricultural soil.

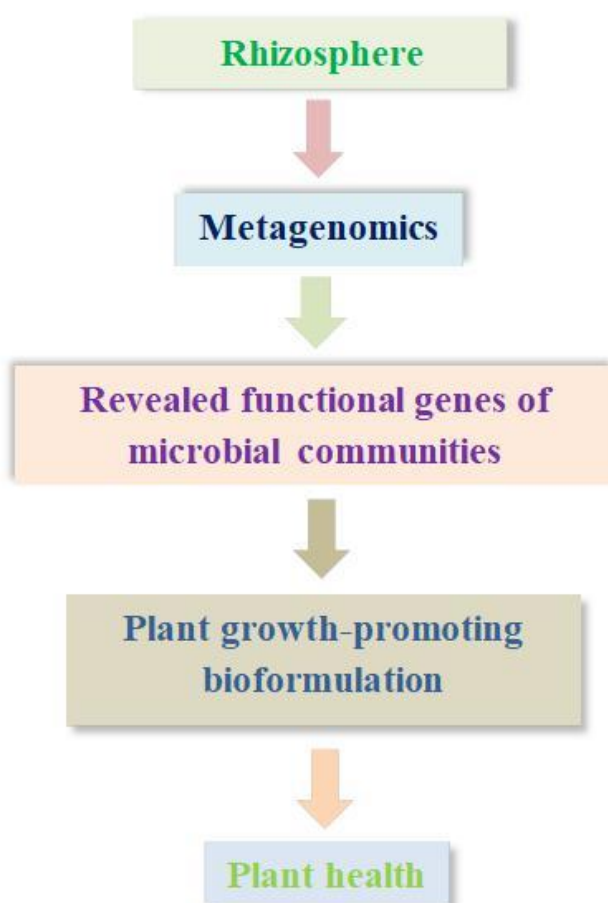
Crops studied	Sampling site	Nature of soil and its characteristics	References
Soybean ( <i>Glycine max</i> L)	Free State, South Africa	Sandy and almost neutral pH	[71]
Maize ( <i>Zea mays</i> L)	Mahikeng, South Africa	The soil has 85% sand, 13% clay, and an average mean of pH 5.93	[72]
Sunflower ( <i>Helianthus annuus</i> )	Lichtenburg, South Africa	The soil has neutral pH	[73]
Tomato ( <i>Solanum lycopersicum</i> L.)	Mafikeng, South Africa	Loamy and pH of .6.5	[66]
Wheat ( <i>Triticum aestivum</i> )	Cameroun, Senegal	Silt, clay, and loamy with pH ranging from 4.5–5.7	[74]
Coffee ( <i>Coffea arabica</i> )	southern, western, and southwestern parts of Ethiopia.	The soil pH ranges from 4.3–8.2	[75]
Banana ( <i>Musa</i> species)	(Meru, Rombo, and Rungwe) Tanzania and Uganda (Port Fortal, Kyenjojo, and Luweero)	Clay loam, pH Tanzania 7.4 and Uganda 6.8	[76]
Sorghum ( <i>Sorghum bicolor</i> )	Ethiopia	The soil pH ranges from 5.9–7.7	[77]
Moringa ( <i>Moringa oleifera</i> )	Ilorin, Nigeria	Sandy	[78]
Crop rhizosphere	Maputo, Mozambique	Sandy, alkaline pH (8.1–8.4)	[79]
Maize ( <i>Zea mays</i> ), rice ( <i>Oryza sativa</i> ), and wheat ( <i>Triticum aestivum</i> )	Kaduna and Kano, Nigeria	Sandy in nature	[80]
Pigeonpea ( <i>Cajanus cajan</i> ) and white yam ( <i>Dioscorea rotundata</i> )	Fumesua and Ejura, Ghana	Fumesua (Greyish brown sandy clay loam topsoil, acidic pH 4.4–5.2) and Ejura (Sandy loam, alkaline pH 6.6–7.9)	[81]



### 3. Concept of metagenomics in tomato rhizosphere microbiome and its relationship with host plants

The metagenomic technique of the tomato rhizosphere soil community displaying the microbe's analysis present in the soil is made up of bacteria, fungi, archaea, and actinomycetes, as observed in Table 2. These microbes are confirmed in a rhizospheric soil sample, particularly on the DNA sequencing method and function of the gene found in the soil [27].

Metagenomics enhances knowledge and understanding of the microorganisms' significance to plants and their interaction, as explained in Figure 3. Moreover, there are different bioinformatic tools employed in metagenomic sequencing of which the following listed were applied in the analysis of sequences; PICRUSt, Tax4Fun, and shotgun metagenomic sequencing.



**Figure 3.** Flowchart displaying the metagenomics studies of rhizosphere microbiomes.

The first tool produced for functional prediction from sequences obtained from the 16S marker is PICRUSt. It is mostly employed for sequencing but due to some challenges faced, it was not the best tool for metagenomic sequencing. Employing 16S rRNA sequence of genes, standard PICRUSt1 helps to predict Kyoto Encyclopedia of Genes and Genomes (KEGG) Ortholog functions [82]. This tool derives the content of unknown genes by a prolonged state of the ancestral reconstruction algorithm in

the employed rhizosphere of tomato according to [83]. The algorithm employs the application of the phylogenetic tree of Sanger sequencing (16S rRNA gene sequences) as conducted by [84] in the rhizosphere of tomato to connect gene content to the operational taxonomic units (OTUs) [85]. The procedure of the tool includes supplying sequences to become produced OTUs from closed-referenced OTU-picking against congenial forms of the greenhouse database [86]. As a result of the limitations of the OTUs, PICRUSt1 progression is incompatible to sequence denoising processes, which liberate amplicon sequences variants (ASV) compared to OTUs. ASVs have better resolution, which allows the differentiation of related microorganisms.

Another novel metagenomic tool for profiling the functional diversity of the microbial community inhabiting samples based on 16S rRNA data is Tax4Fun and employed to unveil the functional diversity in the rhizosphere of tomato plants [87]. The connection of the functional annotation of prokaryotic genome sequences with 16S rRNA gene sequences is identified with closely related genomes based on the sameness of the limited 16S rRNA sequences [82]. This tool can also be used to obtain 16S rRNA procedure pipelines that can carry out the functions of the 16S rRNA gene reads to SILVA [88]. The effect of this tool reveals that the relationship between predicted functions and the metagenome is greater, unlike the PICRUSt tool.

Shotgun metagenomic sequencing (MGS) is the best sequencing tool due to its potential to reveal the entire microbial diversity, functional diversity, and microbial genomes, unlike the gene marker that can be only used to characterize microbial function in a given rhizosphere soil sample [89]. Few studies have reported how the shotgun approach has been used to unveil genomic sequences [90], microbial diversity [3], and functional diversity [66] in tomato rhizosphere. The disadvantage of this sequencing tool is that it does not execute its operation well in case of little microbial community or host contamination e.g. in the biopsy.

In the ecosystem of the developing tomato plants, the plant inhabits various microbiomes as reported in Table 2 to contribute to the health status, plant growth promotion, antagonistic activity against phytopathogen, etc. The microbial communities' diversity is highly underestimated below 5 % microbial population is culturable. The plant-associated microbes are made up of beneficial microbes including plant growth-promoting rhizobacteria (PGPR) [70,91,92], fungi (PGPF) [93–95], actinomyces [96–98], archaea, etc. the following bacteria including; *Pseudomonas*, *Burkholderia*, *Bacillus*, *Azobacter*, *Bradyrhizobium*. Other fungi like *Trichoderma*, *Penicillium*, *Aspergillus*, Arbuscular mycorrhizae, etc., and archaea have been reported to reveal plant growth traits in the rhizosphere of tomato plants. Some of the listed microbes have shown the potential to solubilize phosphates, siderophore production, and indole acetic acid production [96,97]. Studies have also reported that rhizosphere microbiomes can control abiotic stresses [99–101] among which chelating of heavy metals, and uptake of trace elements (Fe and Zn). More of the microbial activity in the rhizosphere of tomato were presented in Table 2 below.

**Table 2.** Microbiomes and their activities in the tomato rhizosphere soil.

Microbiome	Species	Activities	References
Bacteria	<i>Burkholderia contaminans</i>	The PGPR has been reported to inhibit and or control the invasion of <i>Fusarium</i> wilt on tomatoes	[91]
	<i>Burkholderia</i> spp. and <i>Pseudomonas</i> spp.	The PGPRs carry out antifungal potential on tomato plants reducing the growth of phytopathogens and improving the health status of tomato plant growth under salinity conditions	[102]
	<i>Pseudomonas</i> or <i>Bacillus</i> spp.	These bacterial species antagonize <i>Ralstonia solanacearum</i> causing wilt disease in tomato	[70]
	<i>Chenopodium quinoa</i>	The rhizosphere bacteria was reported to induce resistance against <i>Alternaria alternata</i> in tomato	[103]
	<i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Acidobacteria</i> , <i>Bacteroidetes</i> , <i>Chloroflexi</i> , <i>Gemmatimonadetes</i> , and <i>Firmicutes</i>	The following phyla were reported to promote tomato plant growth in greenhouse soils under long-term tomato monoculture	[104]
	<i>Bacillus subtilis</i> , and <i>Bacillus amyloliquefaciens</i>	The PGPR was used alone and in conjunction with cyanobacteria to improve the quality and production of tomatoes	[92]
	<i>Bacillus subtilis</i>	Green waste biochar and wood biochar, together with compost and PGPR against early blight diseases of tomatoes caused <i>Alternaria solani</i>	[105]
	<i>Enterobacter</i> spp. Strains, <i>Paenibacillus</i> spp. Strain, <i>Enterobacter mori</i> strain Lelliottia spp. Strain	PGPR was reported to reduce the stresses on tomato plants through metabolomic analysis	[99]
	<i>Bacillus</i> spp. and <i>Pseudomonas</i> spp.	The PGPR in addition to spent mushroom substrate promotes tomato plant growth	[106]
	<i>Streptomyces cinereoruber</i> strain P6-4, <i>Priestia megaterium</i> strain P12, <i>Rosellomorea aquimaris</i> strain P22-2, and <i>Pseudomonas plecoglossicida</i> strain P24	They involve in plant growth-promoting activities like the production of IAA, phosphate solubilization, and siderophore production	[96]
	J-62 strain	The combination of compost, biochar, and PGPR produced significant results in promoting tomato plant growth, because Cd metals were found in the soil in a combined state, and the growth of tomatoes was sustained by the PGPR in the presence of these metals	[107]
Fungi	<i>Arbuscular mycorrhiza</i> fungi (AMF) ( <i>F. mosseae</i> and <i>G. fasciculatum</i> )	These fungi are efficient biological control organisms against tomato diseases	[108]
	<i>Penicillium cyclopium</i> , <i>Trichoderma harzianum</i> and <i>Trichoderma pseudokoeningii</i>	These rhizosphere PGPF act against <i>Fusarium oxysporum</i> causing soft rot in tomatoes	[94]

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Microbiome	Species	Activities	References
	<i>Purpureocillium lilacinum</i>	This nematophagous fungus has the potential to control the invasion of <i>Nacobbus aberrans</i> and contribute to growth promotion in tomato plants	[109]
	<i>Penicillium</i> spp., <i>Aspergillus</i> sp., <i>Coprinellus radians</i> , <i>Neurospora</i> spp., <i>Paecilomyces</i> spp.	The PGPF aids the germination of tomato seedlings when combined with silicate	[110]
	<i>Epichloë typhina</i> and <i>Curvularia protuberate</i>	Improve the negative impacts of biotic and abiotic stresses, such as heavy metal toxicity, oxidative stress, drought, salinity, and extreme temperatures on tomato	[101]
	<i>Metarhizium anisopliae</i>	The fungi promote the growth of tomato, maize, and arabidopsis	[111]
	<i>Serendipita indica</i> and <i>Serendipita herbamans</i>	The fungi endophytes contribute to tomato plant growth and development	[112]
	<i>Rhizophagus irregularis</i>	The fungi contribute to the growth promotion and quality of tomato plant	[113]
	<i>Xylaria feejeensis</i>	The PGPF biologically controls tomato disease (Early Blight and Fusarium Wilt) and endeavor plant growth promotion	[114]
Archaea	<i>Haloterrigena</i>	The archaea contribute to growth promotion and improve the health status of tomato plant	[3]
	Thaumarchaeota and Euryarchaeota ( <i>Methanosarcina</i> , <i>Methanoculleus</i> )	The archaea were reported to be abundant in the rhizosphere of tomato plants	[115]
	Euryarchaeota, Crenarchaeota, Thaumarchaeota, and unclassified Archaea	The archaea were reported to be abundant in the rhizosphere of tomatoes thereby promoting the health status of tomato plants	[3]
	<i>Methanosaetaceae</i> , <i>Methanosaetaceae</i> , <i>Methanosaetaceae</i> , and <i>Methanobacteriaceae</i>	The archaea are L-AD effluent and very effective inoculum for SS-AD of on-farm organic waste (ie. co-digestion of tomato residues)	[116]
Actinomycetes	<i>Janibacter</i> spp.	Promote tomato plant growth	[117]
	Ac83, Ac4, Ac9, Ac70, and Ac33	The actinomycetes were observed to inhibit the growth of <i>Botrytis cinerea</i> in vitro confrontation and were tested for biocontrol potential on tomato	[118]
	<i>Streptomyces</i> spp., <i>Streptomyces atratus</i> , and <i>Arthrobacter humicola</i>	Promote the growth of tomato seedlings, and induce resistance against phytopathogens <i>Sclerotinia sclerotiorum</i>	[119]
	<i>Methanosaetaceae</i>	They are the dominant methanogens employed in treating tomato waste	[116]
	<i>Streptomyces albidoflavus</i> H12 and <i>Nocardiopsis aegyptica</i> H14	These strains revealed in-vitro antagonistic activities of soil-borne pathogens of tomato plant	[98]

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Microbiome	Species	Activities	References
	<i>Nocardiopsis dassonvillei</i> .	The archaea are involved in various activities thereby improving tomato growth like; dissolving inorganic phosphate, producing ammonia, producing HCN	[97]
	<i>Streptomyces iakyrus</i> .		
	<i>Streptomyces xantholiticus</i> .		
	<i>Streptomyces xantholiticus</i> .		
	<i>Streptomyces albidoflavus</i> .		
	<i>Nocardiopsis aegyptica</i> .		
	<i>Streptomyces thinghirensis</i> .		
	<i>Streptomyces anulatus</i> .		
	<i>Nocardiopsis alba</i> .		
	<i>Streptomyces ambofaciens</i> .		
	<i>Streptomyces xantholiticus</i> .		
	<i>Streptomyces thinghirensis</i> .		
	<i>Nocardiopsis aegyptica</i> . and <i>Nocardiopsis dassonvillei</i>		
	<i>Streptomyces</i> spp. NBM3, <i>Streptomyces</i> spp. NBM2, <i>Streptomyces</i> spp. NBM1, <i>Streptomyces</i> spp. NBM12 and <i>Streptomyces</i> spp. NBM8	The archaea biologically control fungal disease caused by <i>Rhizoctonia solani</i> on tomatoes. The microbes are further known to produce indole acetic acid (IAA), and siderophores and promote tomato plant growth	[120]

### 3.1. Metagenomic of rhizobiome

Nannipieri et al. [121] reported various similar libraries in metagenomics. These include DNA, eDNA, and zoo libraries. Metagenomic research has focused on minor forms of microbial communities. Detailed experience with microbial communities and their performance should be continuously useful for progress in such techniques.

The microbiome community in the rhizosphere soil is a significant source of biomolecules applied in agricultural practices and medical fields (antibiotic medicines, immune suppressors, and anticancer agents). Metagenomics displays a tremendous amount of 16S rRNA gene sequences found in particular bacteria of uncultured species [122]. With the assistance of metagenomics, which provides information on features of specific genes, many microbes, and microbial genes have been identified.

### 3.2. Similarities and differences between the functional diversities in rhizosphere soils

Microbial functions embedded in the rhizosphere of plants occur in the manifold. They involve the breakdown of metabolic substances that improve nutrient production and other needed materials needed for plants' growth [123]. Various functional metabolisms include; protein metabolism, phosphorus, potassium, and sulfur cycling, in the plant rhizosphere that promotes the health condition of the plants [124].

Functional diversity is the component determined by microbial ecologists as the significant link between biodiversity with the activities of microorganisms in their ecosystems. It assists in the determination of the trophic relationships between microbes, their engagement in biogeochemical

cycles, and their responses to environmental change. These functions in the rhizosphere soil are abundant and as a result, improve the fertility and quality of the soil [125]. Some studies have presented functional analyses associated with different crops and there are similarities between the functions presented. Adedayo et al. [66] presented 15 functional diversity to be greater in the healthy rhizosphere of the tomato plant compare to the diseased tomato plant rhizosphere and they include; Carbohydrates, Miscellaneous, Cofactors, Vitamins, Prosthetic Groups, Pigments, Cell Wall and Capsule, Nucleosides and Nucleotides, Fatty Acids, Lipids, Isoprenoids, Clustering-based subsystems, Stress Response, Nitrogen Metabolism, Regulation and Cell signaling, Motility and Chemotaxis, Cell Division and Cell Cycle, Potassium metabolism, Dormancy and Sporulation, and Photosynthesis. Other studies presented various functions including cell wall and capsule, protein metabolism, regulation and cell signaling, iron acquisition and metabolism, membrane transport, miscellaneous, motility and chemotaxis, nitrogen metabolism, potassium metabolism, RNA metabolism, stress response, phosphorus metabolism, sulfur metabolism, and virulence, disease and defense in maize rhizosphere as reported by Chukwuneme et al. [126]. Most of these functions were also reported according to Ajiboye et al. [71] that the functional diversity including nitrogen metabolism, sulfur metabolism, iron acquisition, etc is more abundant in the rhizosphere of soybean compared to bulk soil. The studies mentioned above employed the shotgun metagenomic sequencing approach to unveil these functions. The introduction of a culture-independent approach especially shotgun metagenomics has assisted in subjugating this limitation.

### 3.3. Microbial genome in rhizosphere soil

The genomic technology of next-generation revealed comparative genomics of phytopathogens and their biomass [127]. This method has been applied to determine genes associated with a significant disease or phenotypic immunities like type III secreted effectors (T3SEs) produced by Gram-negative bacteria and certain groups of effectors in fungi and oomycetes [128]. Bacterial pathogens of comparative genomics have shown some pathogens can utilize the same process to bring down the immunity of host plants which is accomplished via different evolutionary routes [129]. A typical example obtained in the independent lineage of *Pseudomonas syringae* is to destroy the immune response of host plants. An organism may share more than half a percentage of its genome with other organisms of the same species [130]. Yet the total genome of the organism can be more than 10 times more than the size of the individual genome. Comparative genomics showed the adaptation of genomes that are related to the nature of the specific pathogen, with multiplication and diversification of a group of genes encoding enzymes required for the lysis of the cell wall of the organism and biological synthesis of secondary metabolite to manufacture some materials that possessed host cytotoxic potential [131]. Common characteristics of necrotrophic bacteria and fungi are their potential adaptation. In comparison, the characteristics of obligate phytopathogens show how the organisms derive nutrients from the living host due to the declination of the convergent gene in metabolic tracts[132]. This supposedly explains the reason why phytopathogens become obligate parasites. Culture-independent methods together with metatranscriptomics studies unveil the knowledge of implementing plant-microbial interaction with specific taxonomic diversity [133]. Investigating the major microbial functions reveals mutualistic and commensal groups that require a

statistical design with some replicates, the formation of synthetic model communities that can be influenced, and the interpretation of multiple omics data types.

Beneficial organisms that include arbuscular mycorrhizal fungi, chlamydia, macroinvertebrates, nitrogen-fixing bacteria, parasites, and protozoa, exist and compete for available nutrient in the rhizosphere soil that contributes to the growth of the plant. For the translocation of minerals, mycorrhizal fungi are symbionts that play an important role in this process [134], they can inhibit soil-borne spoilage organisms of plants [135], and fixes nitrogen by biological means [136]. Soil microorganisms can regulate the plant's immune systemic resistance by modifying pathways that include various defense genes (*MPK3* and *MPK6*), and the ethylene or jasmonic acid pathway [137].

Liu et al. [138] reported how Phosphomonoesterases are the significant enzymes in the mobilization of Phosphorus (P). The functional gene (*phoD*) and the soil pH are responsible for the control of the procedures of alkaline phosphomonoesterases (ALP) [139]. As a result, ALP processes have not been confirmed in plants, it was observed that the genes that code for ALP in soils are found in soil bacteria families that include *Methylobacteriaceae*, *Bradyrhizobiaceae*, *Methylococcaceae*, *Burkholderiaceae*, *Rhodobacteraceae*, and *Tolypotrithaceae*. *PhoD* gene is abundant in rice paddy soil that contains low P conditions and improves operational taxonomic units of *Actinobacteria* and *Cyanobacteria* [140]. The functional genes abundance and various activities of the enzymes were not importantly corresponded when the P required by microorganisms and plants were acquired [141]. Yet, the abundance of *phoD* genes was positively variable with the process of ALP after the application of manure in an organic agricultural farm [142]. So, the expression of this functional gene in soil revealed the process of mineralization of P is being controlled in the region corresponding to rhizosphere soil [143]. This reveals how microbial communities and crop plants endure during the growing phase amidst P deficiency in soil with low alkalinity [144].

#### **4. Prospect: modern approaches for assessing functional plant microbiome**

This study adds more quality to the assessment of microbial communities in the rhizosphere soil of tomatoes. It then explains how metagenomics methods in high-throughput next-generation sequencing technologies have helped eradicate hunger among the populace and improve the abundant production of tomatoes in the food security sector.

According to metagenomic studies, the number of catalytic purposes embedded in the microbiota far outnumbers the plant's catalytic capacity [145]. Any microbiota functions may directly improve plant output, with three distinct pathways receiving the most attention. They include plant nutrition improvement, pathogen outbreaks curtailment, or the nonliving factor endurance modulation [146]. Selective promotion of these beneficial microbial activities in the agricultural system may be a way to improve crop yields while reducing synthetic fertilizer and pesticide use.

Integrating microbiome science into farm practice can significantly increase agricultural sustainability. Thus, specific significant motivation gaps limit our potential to influence the microbiota found in plants [147] logically. Crop varieties were selected based on their capacity to attract advantageous microorganisms into the soil at the root region. It referred to one way of encouraging favorable plant-microbe interactions [148]. However, this technique is not yet commercially viable due to the exclusion of high-throughput screening methods for the physical composition of microbial-mediated characters in high-fostering communities and an insufficient understanding of how genetics

in plants impacts the microbiome [149,150]. Inoculating crop plants with advantageous microbes is another alternative. Future studies should aim to determine how the complex plant microbiome interaction affects final crop yield and, as a result, to modify the effectiveness of microbiome-based agricultural adjustments.

## 5. Conclusion

Microorganisms that live in the soil are at the root region of the plant, thereby supplying double environments in a different compartment of the plant for reproduction. As a result of plant development, soil microbes are subjected to some status as plants react to changing conditions related to physiological modifications. Over the last few years, we have learned much about microbial population dynamics in different plant microenvironments. However, we still do not know which physiological conditions (microbiomes, plant microbial, and environmental conditions) favor the plant species' growth. We also gained knowledge on the potency of plant-associated microbiome metagenomic activities. Nevertheless, the operation acted in plants is unfortunately comprehended, especially regarding the variety to which microorganisms are exposed and the importance of the genes produced. We will have to acquire more knowledge of plant reactions to rhizosphere soil microbiota, environmental status, and an appreciative understanding of the molecular interactions between microbiomes and plants. There is a need to focus on microbiome progress from studying microbiota structural composition to comprehending functional aspects.

## Conflict of interest

The authors declare no conflict of interest.

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