

Research Article

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Biofertilizer Impacts on Cassava (*Manihot Esculenta* Crantz) Rhizosphere: Soil Microbiome Engineering, Genetic and Sustainable Agroecosystems, Igbariam, Nigeria

Otaiku AA^{1*}, Mmom PC² and Ano AO³

¹Doctoral student, Faculty of Social science, Department of Geography and Environmental Management, University of Port Harcourt, Choba, Port Harcourt, Rivers states, Nigeria

²Faculty of Social science, Department of Geography and Environmental Management, University of Port Harcourt, Choba, Port Harcourt, Rivers states, Nigeria

³National Root Crops Research Institute, Umudike Umuahia, Abia state, Nigeria

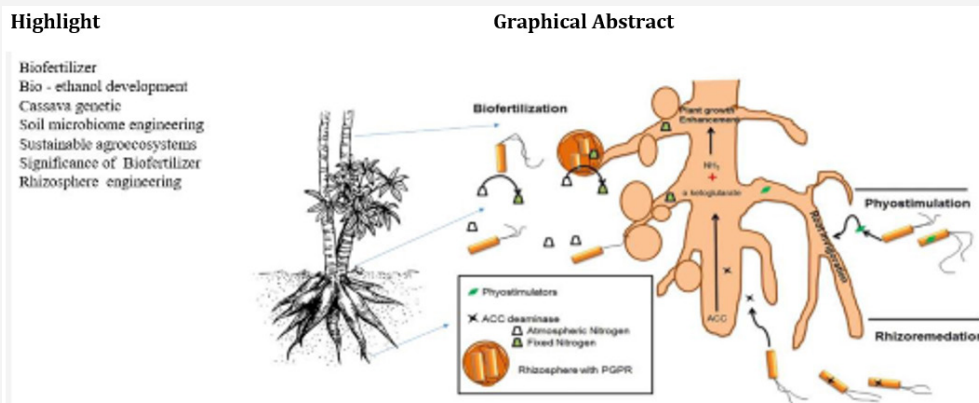
***Corresponding author:** Otaiku AA, Doctoral student, Faculty of Social science, Department of Geography and Environmental Management, University of Port Harcourt, Choba, Port Harcourt, Rivers states, Nigeria.

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Abstract

There is a wealth of unexplored knowledge about microbe's ecosystem functioning impacts on food security, climate change mitigation and sustainable agriculture with focus on cassava cultivation in the tropics using biofertilizer. The rhizosphere modified by biofertilizer as agronomic management during cassava cultivation by the inoculants for microbiome engineering for integrated soil fertility management by the release of plant root exudates and microbial metabolites. Microbiome and the cassava crop are highly dependent on each other as the microbiome contributes a significant portion of the secondary genome of the host plant like quorum quenching strategies that suppress the virulence of pathogens, enhance high yielding cultivars and favorable environments for development. Rhizosphere engineering using biofertilizer can reduce and improves chemical dependant agriculture for resilience agriculture. Cassava genetic engineering integrated with biofertilizer can transform the use of cassava cultivar for polluted soils phytoremediation and bio-energy crop cultivation in the tropics as re-generative agriculture technique for xenobiotic pollution management. The economic impacts will be development of remediation-to-biofuel economy of restorated polluted soils by conversion of the bioenergy crop cultivated into bioethanol. Cassava cultivation with biofertilizer accentuate regenerative agriculture as an integral potential for food security and sustainable development goals (SDG).



Keywords: Biofertilizer; Rhizosphere engineering; Cassava genetics; Niger-Delta; Sustainable development goals (SDG); Biodegradation; Remediation-to-biofuel development; Re-generative agriculture; Bio-ethanol

Introduction

Currently, researchers round the globe are facing challenges of maximizing the functions of microbiome under the limitation of the natural and anthropogenic activities including new strains of pests and pathogens, climate change, use of chemical fertilizers, as these activities are continuously menacing stable agricultural productions [96]. The agriculture paradigm shifts in 21st century is re-generative agriculture techniques by deploying the soil microbiome for integrated soil nutrient management as affirmed by scholars [97,627,703] and to enhance plant nutrient uptake. Soil microbiome represent an unexploited pool of opportunities to face the sustainability issues of agriculture [69,93,143,257,507] under climate change. The intricate interactions between the plant genotypes, microbiome structures and different environmental factors offer indispensable information in sustainable agriculture. The research articles are trilogy: Biofertilizer Impacts on Cassava (*Manihot esculenta Crantz*) Rhizosphere: crop yield and growth components (paper 1); improved soil health and quality (paper 2); soil microbiome engineering, genetic and sustainable agroecosystems (paper 3). The crux of the paper is that “the agriculture production and sustainability is under threat” from various natural and anthropogenic factors, farm inputs, agronomic practice and plant-microbe’s interactions that sustains the earth biodiversity”. Biofertilizer enhances soil microbiome techniques of plant-microbe interactions.

Soil is the most diverse and complex habitat that consists of millions of fungi, billions of bacteria and other macro organisms [55]. Microorganisms present in soil play important roles in nutrient cycling and shielding plant from harmful effects of abiotic and biotic stresses [12,13,247,310,317]. Intensive agriculture practices lead to increase in crop production but in the same time, it poses detrimental effects on the biological and physical properties of soils. In agricultural systems, macronutrients are generally provided through chemical fertilizers and a treat to modern agriculture and compared to applications of biofertilizer and biopesticide that improve the growth of plants health, crop productivity and enhance biodiversity. Plant growth promoting rhizobacteria, a group of diverse rhizosphere microbe, produce a variety of bioactive chemical substances that besides promoting the growth of plants, protect the plants from pathogens [157]. Microbes, the most diverse and profuse group of organisms constitute more than 60% of the Earth’s biomass [57] sustains the vibrant integrity and equilibrium of biosphere is imperative, as the subsistence of life is reliant upon the sustained and microbial arbitrated transformation of matter, both in the aquatic as well as terrestrial environments [652]. Microbes have a myriad of functions, and they play an imperative role in sustainability and biogeochemical cycling [132]. This cycling of elements besides shaping the structure and function of ecosystems also enriches the soil with the abilities that can provide varied services to the people [15]. Soil microbiome along with their allied functions determines the productivity of agro-ecosystems

[625], sustainable agriculture relies on soil health, diversity of microbes for soil biodiversity. It is estimated that majority ($\geq 90\%$) of the microbial diversity still remains to be explored. These novel unexplored diversities correspond to treasure troves of improved and innovative biotechnological developments with applications in the fields of energy, agriculture, chemicals, mining, materials, food, pharmaceuticals and environmental protection. Identification of the key ecosystem driving elements is one of the challenging tasks and manipulating these drivers to produce appropriate benefits is even more demanding for modern agriculture today.

Soil microbiome interactions involving plants and roots in the rhizosphere include root-root, root-insect, and root-microbe interactions. The root microbiome is the main determinant for the plant growth and health; and does so, by assisting the host plant in nutrient uptake, protection against pathogen attack and by supporting abiotic stress tolerance [69,90,157,527].

Synchronized interactions between the microbes and their host plants encompass a supreme importance and significance for improving the plant growth and in maintaining appropriate soil conditions. There is now overwhelming evidence which supports the fact that plant can shape their microbiome by the belowground plant microbe interaction [103].

Even the most ancient lineages of plants show a strong ability to alter the relative abundance of microbial groups in the soils surrounding the rhizosphere [621]. The close symbiosis of plants and microbes can be viewed as an integrated ecological unit known as a halobiont [632]. These contrasting microbiomes have been attributed to differences in root exudate chemistry [46,498] and in plant nutrient uptake rates [62]. Genotypic and phenotypic variations in plant traits that support microbiomes that increase plant nutrient availability prevent pathogens or otherwise enhance plant health, growth and performance incur a fitness advantage, now affirmed by omics tools of biotechnology for modern agriculture.

Cassava Nutritional Health

The exponential growth in human population has demanded a concurrent production and supply of food, particularly from plants. Consequently, a highly productive and intensive agricultural system has been mostly accomplished by the use of synthetic chemical fertilizers of nitrogen and phosphorus [534] resulting to environmental pollution problems by emissions of greenhouse gases like nitrous oxide (N_2O) from fertilizer production and application [411]. A biofertilizer of selected efficient living microbial cultures, when applied to plant surfaces, seed or soil, can colonize the rhizosphere or the interior of the host plant and then promote plant growth by increasing the availability, supply, or uptake of primary nutrients to the host. Biofertilizers are mostly supplied as conventional carrier-based inoculants in liquid or solid forms. The mass production of biofertilizers involves culturing of

microorganisms, processing of carrier material, mixing of carrier material with the broth culture, and packing (focus of paper 1) and it is predicted that market share of biofertilizers will reach US\$1.66 billion by 2022 and will be compounding the annual growth rate of 13.2% during the years of 2015–2022 [396,602] SCOPE 65 reported. Okon & Labandera-Gonzalez [435] were firstly arguing that rhizospheric organisms which improve soil nutrients utilization. Fuentes-Ramirez & Caballero-Mellado [199] defined biofertilizer as “a product that contains living microorganisms, which exert direct or indirect beneficial effects on plant growth and crop yield through different mechanisms”. Vessey [651] affirmed, biofertilizer are formulated product containing the microorganisms that is applied to the plant or soil. Indeed, it has been proved that consortia of species normally improving nutrient efficiency (e.g. double inoculants with bacteria and fungi in one gel formulation) can show plant protection properties [639-641,651].

Climate change also greatly impact upon overall quality of the crop and the dynamics of the associations that exist between crops, pests and diseases. Fluctuations in climatic factors like rainfall, solar radiation, and temperature have great potentials to influence crop production [120]. Various soil microbes interact with each other as well as with plants in a myriad of different ways that help in maintaining and shaping different components of ecosystem [70,249,477]. These interactions have great potential to mediate some very important processes like composition of plant community, mineralization, and shifts in ecological interaction related important functions [6,279]. Climate change alters plant phenology and distribution of microbes therefore, plant species distribution is affected in response to climate change [120]. The unsustainable use of chemical fertilizers is causing the disruption of Earth's bio-geochemical cycles by altering the mechanism and are responsible for soil degradation, eutrophication, and greenhouse gas emissions [22,578].

Production of N-fertilizer using energy-intensive Haber-Bosch process relies upon fossil fuels and thus contributes to global warming and natural resource depletion which ultimately contributes to climate change [174]. Chemical fertilizers application induces severe consequences, alternative methods for sustaining soil health and plant nutrition with minimum input of mineral fertilizers is needed [192] - the paper objective for alternative resilience agriculture with beneficial and specific root-associated microbes that mineralize the bound organic nutrients for enhanced biodiversity. There is vast assemblage of microbes (fungi and PGPRs) that colonize the plants roots and provide ecological services for increment of plant diversity, enhancement of seedling recruitment and better nutrient acquisition. Biofertilizer supplies functional diversity of symbionts for root microbiome to complement each other each other in obtaining various limiting nutrients and in driving ecosystem functions and affirmed by scholars [626,659].

Rationale

Recently the field of plant biology has recognized the importance of root exudates in mediating these biological interactions [39]. Identification of the key ecosystem driving elements is one of the challenging tasks and manipulating these drivers to produce appropriate benefits is even more demanding. The impact of a warming climate on spring plant phenology is evident [20,78].

A longer growing season may increase carbon uptake and potentially mitigate climate change [78,154], leaf emergence [283], fruiting [684], and germination [441]. Abiotic stress factors include extreme temperature [357], drought, water logging, light, and salinity as major parameters that affect plant growth and detailed mechanisms are unclear. Plants are immobile, they have coevolved with microbes and acquired a number of mechanisms that modulate the outcome of their interactions [437]. Campbell et al., [98], observed that the density of microbes in the rhizosphere was 100 times greater than that in the bulk soil and plant root exudates shape the soil bacterial community [242,330]. How does the microbiome diversity function potentially affect host plant performance? The presence of microbial hubs in plant microbiome networks plays an important role between a plant and its microbial community [624] with key microbial metabolic processes related to plant nutrition in paper 1 [448]. The mechanism of action of Plant growth-promoting rhizobacteria (PGPR) in the biofertilizer applied to cassava cultivation (paper 1) can produce a complex blend of volatile substances, which are distinct between bacterial species and other closely related species [214,215,233] provides the cassava soil and microbiome engineering. Some of these bacterial volatiles can stimulate plant growth [464,516], suppress disease stimulating ISR [464] or antagonize phytopathogens [290,695], nematodes, or insects [84,87]. Soil resources can also be transferred by shared symbiotic fungi called common mycorrhizal networks (CMNs) affirmed by Simard et al., [555].

How does cassava plant harbor unique microbial communities that shape a unique rhizosphere microbial community is the crux of paper 3? Fungi, PGPR and beneficial microbes in the biofertilizer applied was the methodology to study soils fiction when biofertilizer is applied to the cassava crop cultivation. Scholars affirms that microbes can enhance nutrient uptake, stimulating root, and shoot growth by producing indole acetic acid [260,452], 1-aminocyclopropane-1-carboxylate (ACC) deaminases [221,222], solubilizing phosphate [223], and enhancing the uptake of nutrients from the environment [53,162]. Microbes can enhance plant resistance to adverse environmental stresses such as drought, heavy metals, salts, and nutrient deficiency [705].

Biotic stress factors include interaction with other organisms and infection by pathogens or damage by insect pests, and some plant growth-promoting bacteria have been used as biocontrol

agents against plant pathogens [262,265]. These are the questions that must be addressed. Modern genomic technologies (e.g., high throughput sequencing) can provide clues to the answer. The paper investigates the plant-microbe interactions at the soil functional level (what they are doing) in order to identify the signals involved in the interspecies interactions, food security and re-generative agriculture using cassava as phytoremediation plant for polluted soils and cultivation bioenergy crop for bio-ethanol production.

In this context of doubling the global food demand by the year 2050, necessitates the expeditious and instant solutions [601] by deploying the soil microbiome to increase the resistance to various stresses (biotic and abiotic) reported by [97,627,703] and to enhance plant nutrient uptake. Hiruma et al., [257] present one of the few unexploited pools of opportunities to face the sustainability issues of agriculture [69,93,143,507] under climate change (Figure 1).

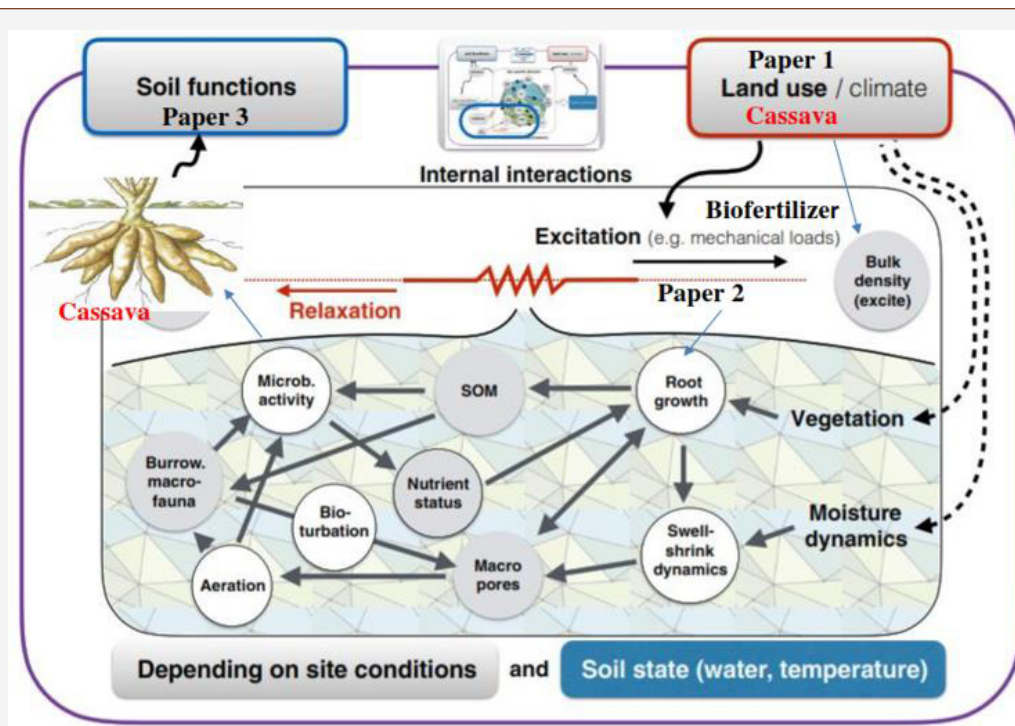


Figure 1: Impacts of 'biofertilizer rhizosphere to cassava trilogy papers schematic' adapted. from Vogel Hans-Jörg et al., 2018.

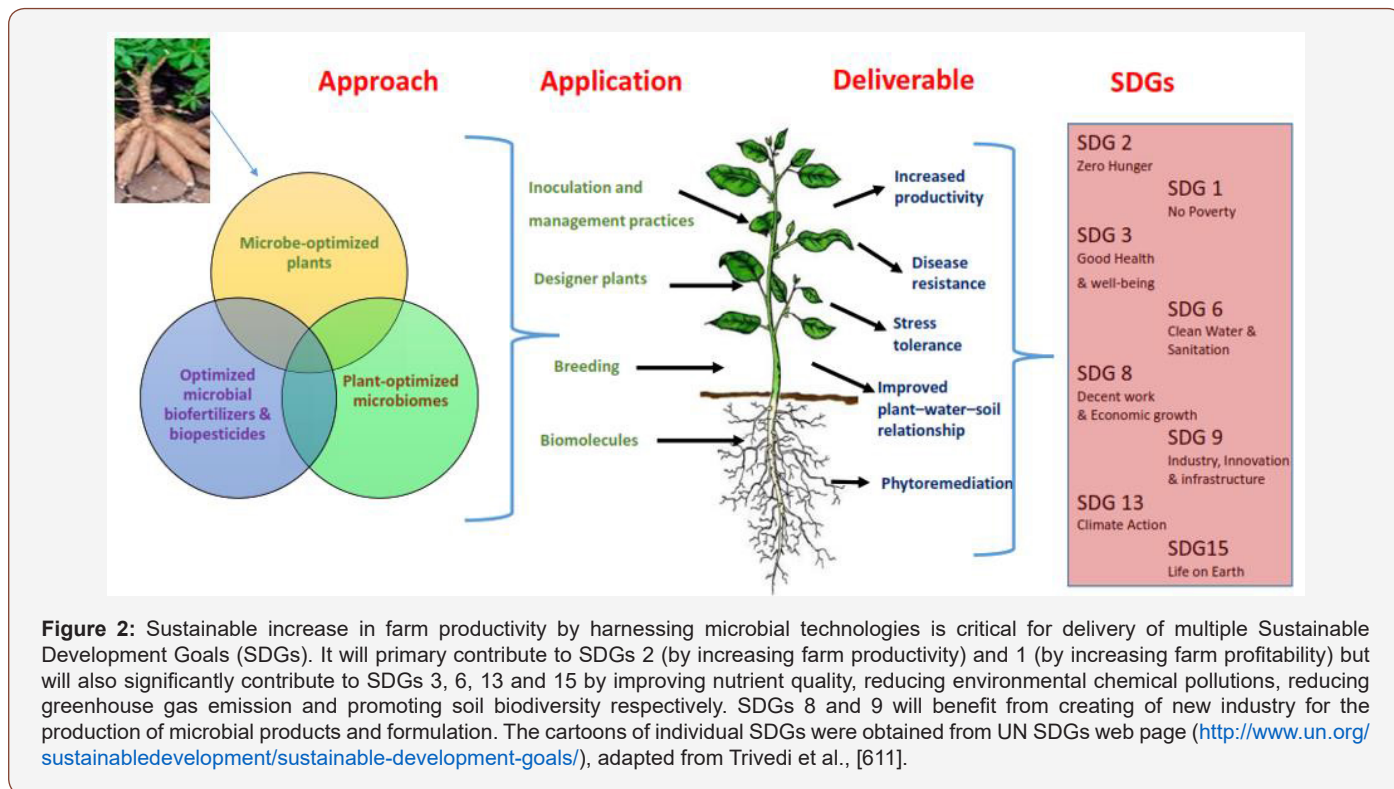
Methodology

Cassava crop and microbes can interact at soil aggregate scale, with substantial variations being noticed across soil aggregates [341]. Synchronized interactions between the microbes and their host plants encompass the role of soil microbiome in crop development and integral function of the microbial inocula in the formulation (PGPRs biosurfactants presents in OTAI AG® Inocula, Otaiku et al., [448]) of the biofertilizer applied during the cassava cultivation, PGPR gains unique and extraordinary attention due to their diverse functional characters such as production of hormone and certain beneficial enzymes, effective root colonization and solubilization of nutrients for sustainable agriculture. Knowledge and understanding regarding the ecology, growth promoting characters, mechanism of action as well as application of the naturally occurring microbial populations hold key importance for plant growth and soil microbiome as alternative to chemical fertilizer. Soil microbial community intimately relate to soil physical properties and immediately affect ecosystem processing, their presence, abundance and diversity have often been proposed as bioindicators of soil health reported by Lu et al., [346].

In Otaiku et al. [448] reported that biofertilizer characteristics (Paper 1 Table 5, pages 6 -7) and biosurfactants (Table 3 pages 8-9) applied in the filed cassava cultivation requires no chemical pesticide because cassava plant-microbes associated lifestyle requires adaptation to several niches, in which different metabolites act as signals for interaction (communication) with the plant and host specific plants nutrient and crop protection as narrated. Plant community dynamics are driven by the microbial mediation of soil resource partitioning and sharing in the "rhizosphere" because key microbial metabolic processes related to plant nutrition as executed using biofertilizer [448]. "Rhizosphere" defined as the soil compartment affected by plant roots [262]. Soil microbes are chemotactically attracted to plant root exudates, volatile organic carbon, and rhizodeposition, and then proliferate in this carbon-rich environment [348]. Plant root exudates differ between plant species, so differences in rhizosphere microbiomes of different plant species are expected [514]; Plant species-specific microbiomes [274,300]. Plants can also shape the microbial community via root exudates.

Root exudates can be categorized as sugars, amino acids, organic acids, nucleotides, flavonoids, antimicrobial compounds, and enzymes [39,514]. The change in the microbial composition generates feedback on the plant relative performance that defines the long-term effects of the soil microbes on their coexistence with that plant species [71,73] and affirmed in Figure 2. The feedback can be of two types; positive plant-soil microbial feedback

reinforces the spatial separation of the microbial communities [17], while negative feedback results in plant replacement, which necessitates recolonization of locally specific roots [72,660,664]. Systematic methods such as genome-wide association studies have enabled us to explore the relationships of plant loci and symbiotic communities in details [251,262].



Microbial hubs might be responsible for mediating defense signals among plants and the effectiveness of biological control agents [624]. The term “Endophytic bacteria” has been proposed for the presence of a kind of hub species that would be a determinant of colonization of widely microbial taxa and number of hypothetical relationships between plant performance and microbial diversity and composition have been proposed [624]. Salinity resistant *Pseudomonas fluorescens*, *P. aeruginosa*, and *P. stutzeri* ameliorated sodium chloride stress in tomato plants, and an increase in roots and length were observed. In the past decade, bacteria belonging to different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Enterobacter* and *Methylobacterium* have been reported to endow host plants under different abiotic stress environments [235]. *P. fluorescens* produces 2,4-diacetyl phloroglucinol, which inhibits the growth of phytopathogenic fungi [293].

Soil Microbiome Engineering

Global food supply must grow sustainably within the context of the ever-increasing competition for natural resources, particularly land and water, and competition for food and biofuel, and by the need

to operate in a carbon- constrained economy [537,596]. Increased anthropogenic activities have major effect on environment [358] as a whole and sustainable agriculture in particular [176]. Increasing the productivity of agricultural land in order to produce more food in an environment friendly ways in the era of changing climate, concept of ‘sustainable intensification’ [118] requires microbes to have a myriad of functions, and they play an imperative role in sustainability and biogeochemical cycling [132].

Soil microorganisms including bacteria, archaea and fungi play a diverse and often decisive role towards the functioning of ecosystem such as driving the cycling of major elements (e.g. N, C and P). This cycling of elements besides shaping the structure and function of ecosystems also enriches the soil with the abilities that can provide varied services to the people [15]. Soil microbiome and allied functions determines the productivity of agro ecosystems [625] and this paper accentuates the role of biofertilizer in soil microbiome engineering.

Climate-changing parameters are well known to affect both the micro as well as macro-organisms (plants) and is a major global problem affecting the life on the planet [121,537] and believed to impart both direct and indirect effects on plant-soil-microbe

interactions [4,79,609], by altering the community structure, relative abundance and function, as the soil community taxa vary greatly in their physiology, growth rates and temperature sensitivity [40,248]. Soil is the most diverse and complex habitat that consists of millions of fungi, billions of bacteria and other macro organisms [55]. Microorganisms present in soil play important roles in nutrient cycling and shielding plant from harmful effects of abiotic and biotic stresses [11,12,13,247,310,317]. Plant growth promoting rhizobacteria, a group of diverse rhizospheric microbe, produce a variety of bioactive chemical substances that besides promoting the growth of plants, protect the plants from pathogens [157]. Plants rely on the propensity of their roots to communicate with variety of microbes. The first step in root colonization is production of chemotactic response towards variety of root exudates released by roots of plants. The different types of exudates released by roots include amino acids and organic compounds [708]. The root microbiome is the main determinant for the plant growth and health and does so by assisting the host plant in nutrient uptake, protection against pathogen attack and by supporting abiotic stress tolerance [69,90,157,527].

Various soil microbes in different biofertilizer formulations in agriculture interact with each other as well as with plants in a myriad of different ways that help in maintaining and shaping different components of ecosystem [70,249,477]. The direct impact of climatic change on function and composition of microbial communities have been extensively reviewed by different researchers [1,107,109,111,250,363] but unfortunately the indirect effects via shifting soil microbe–microbe and plant–soil microbe interactions receive very less study. Soil health deliver a range of ecosystem and agronomic functions and services in order to maintain environmental health and quality, biological productivity, promote plant and animal health [327]. Microbes enhances soil

health, improving water holding capacity, carbon storage, root growth, availability and cycling of essential nutrients, filtering pollutants and also in conservation of biodiversity [294,407] (Figure 2).

In Table 9 functional diversity of symbionts, root microbiome can complement each other each other in obtaining various limiting nutrients and in driving ecosystem functions [626,659]. Chemical fertilizers disruption of Earth's biogeochemical cycles (soil degradation, eutrophication, and greenhouse gas emissions) reported by Amundson et al., [22], Steffen et al., [578]. Understanding potential of soils to sequester carbon by microbes are of fundamental importance [22]. Sustainable agricultural practices capable of generating higher crop yields via multidisciplinary coordination among ecology, agronomy, soil science, genetics, economics and social sciences and also without the full engagement of farmers was reported by scholars [110,318]. Soil microbes are the key components of ecosystem and are responsible for crop yield [157], nutrient cycling and carbon sequestration [67], and environmental restoration [359]. There is a wealth of unexplored knowledge about the role of these microbes in ecosystem functioning and climate change. Scientists [254,610,697] suggested that microbes possess natural ability to capture and sequester CO₂ via different metabolic pathways. Similarly, researchers reported that [237,414,686] in their studies discussed the significance of microbes for mitigating the harmful effect of greenhouse gases. Application of metagenomic approaches to study soil microbiomes could greatly help in understanding and restoring ecosystem functioning, which at present are under severe pressure, as these approaches seem to be instrumental in providing useful knowledge about taxonomic, genetic, and functional aspects of soil microbial taxa [188,557,617] (Tables 1&2).

Table 1: Inoculants in biofertilizers applied for cassava cultivation potential for soil microbiome engineering [459].

S. No.	Biofertilizer Types Group	Micro-organisms	Cassava inoculate	Applications	References
Biofertilizer for micronutrients					
1	N ₂ -fixing biofertilizers	Azotobacter, Nostoc	OBD-Biofertilizer	Biofertilizer	Amutha et al. [24]; Otaiku et al., [448]
a.	Free-living	Rhizobium	OTAI X®	Biofertilizer	Meena et al. [382,383]; Otaiku et al., [448]
b.	Symbiotic	Azospirillum, Gluconacetobacter diazotrophicus	OBD-Biofertilizer	Bioremediation	Jaiswal et al. [280]; Murumkar et al. [400]
c.	Associative symbiotic	Associative symbiotic	OBD-Biofertilizer	Xeonbiotic Biodegrader	Otaiku and Alhaji [442]
2	P-solubilizing biofertilizers				
a.	Bacteria	Bacillus megaterium var. Bacillus subtilis	OTAI AG®	Biofertilizer, Biopesticide	Kumar et al. [313]; Meena et al. [377]
		Bacillus circulans, Pseudomonas striata	OBD-Biofertilizer	Xeonbiotic Biodegrader	Bahadur et al. [42]; Das and Pradhan [478]
				Biocontrol	Otaiku et al., [448]; Otaiku et al., [449]
b.	Fungi				
	P-mobilizing biofertilizers	Penicillium sp., Aspergillus awamori	OTAI AG®	Biofertilizer, Biopesticide	Meena et al. [378,381]; Teotia et al. [592];
			OTAI X®, OTAI AG®	Xeonbiotic Biodegrader	Otaiku and Alhaji, [442]; Otaiku and Alhaji [448,449]

Biofertilizer for macronutrients					
a.	Arbuscular mycorrhiza	Glomus sp., Gigaspora sp., Acaulospora sp.,	OTAI AG®	Biofertilizer	Verma et al. [650]; Kumar et al. [315]
		Scutellospora sp. and Sclerocystis sp.	OBD-Biofertilizer	Mycorrhizosphere	Raghavendra et al. [489]; Zahedi [699]
	Sulphur oxidizers	Thiobocclus thioxidans	OTAI AG®	Mycoremediation	Sharma et al. [547]; Vaid et al. [620]
a.	Zinc solubilizers	Bacillus sp., Pseudomonas sp., Aspergillus niger	OBD-Biofertilizer	Biofertilizer	Sharma et al. [547]; Vaid et al. [620]
b.	Potassium and silicate solubilizers	Pseudomonas sp., Bacillus sp.,	OTAI X®	Xeonbiotic Biodegrader	Nath et al. [412]; Sarkar et al. [526]; Otaiku and Alhaji [448,449]
			OTAI AG®	Bioremediation	Rawat et al. [499]; Yasin et al. [695]
c.	Manganese solubilizers	Penicillium citrinum	OBD-Biofertilizer	Bioremediation	Lovley, [345]; Ehrlich & Newman, [165]
Plant growth-promoting rhizobacteria					
a.	PGPR				
	Bacteria	Pseudomonas fluorescens, Bacillus sp.,	OTAI AG®	Biofertilizer, Bioremediation	Yadav and Sidhu [689]; Saha et al. [520]
		Azotobacter, Klebsiella, Enterobacter, Azospirillum,	OBD-Biofertilizer	Xeonbiotic Biodegrader	Meena et al. [316]; Otaiku and Alhaji., [442]
b.	Fungi				
	Bio filmed biofertilizers	Fungal-bacterial biofilms (FBB),	OBD-Biofertilizer	Biofertilizer, Bioremediation	Hettiarachchi et al., [252]; Amundson et al., [23]
		fungal-rhizobial biofilms (FRB); Bacillus cereus	OTAI AG®	Biocontrol	Verma et al. [649,650]; Meena et al. [374,375]

Table 2: Various organic or inorganic substances produced by plant growth promoting rhizobacteria facilitating resource acquisition to stimu plant growth.

PGPR in the Biofertilizer to Cassava	PGP Traits	References
Rahnella aquatilis	ACC deaminase*	Mehnaz, Baig and Lazarovits, 2010
Acinetobacter sp., Pseudomonas sp.; Enterobacter sp.	ACC deaminase*	Indiragandhi et al., [277] Kumar et al., [319]
Pseudomonas jessenii	ACC deaminase	Rajkumar and Freitas, [493]
Pseudomonas aeruginosa	ACC deaminase*	Ganesan, [213]
Achromobacter xylosoxidans A551,	ACC deaminase*	Belimov et al., 2005
Rhizobium hedysari ATCC 43676	ACC deaminase*	Ma et al., 2003
Pseudomonas marginalis DP3	ACC deaminase*	Belimov et al., 2005
Mesorhizobium loti	ACC deaminase*	Sullivan et al., 2002
Rhizobium leguminosarum	Indole-3-acetic acid	Ahemad and Kibret, 2014
Azotobacter sp.; Pseudomonas sp.	Indole-3-acetic acid	Ahmad et al., 2006; Roesti et al., 2006
Bacillus sp, Paenibacillus sp.	Indole-3-acetic acid	Beneduzi et al., 2008
Rhizobium leguminosarum b. Trifolii ACCC18002	Indole-3-acetic acid	Jin et al., 2006
Streptomyces strains C	Indole-3-acetic acid	Sadeghi et al., 2012
Enterobacter aerogenes NII-0907, Enterobacter aerogenes NII-0929,	Indole-3-acetic acid	Deepa, et al., 2010
Pseudomonas tolaasii ACC23, Pseudomonas fluorescens ACC9,	Indole-3-acetic acid	Dell'Amico et al., 2008
Mesorhizobium loti MP6; Enterobacter sp., Klebsiella	Indole-3-acetic acid	Chandra et al., 2007; De Santi Ferrara et al., 2013
Pseudomonas aeruginosa, Pseudomonas fluorescens, Ralstonia metallidurans	Siderophores	Braud et al., 2009
Proteus vulgaris; Enterobacter sp.	Siderophores	Rani et al., 2009; Kumar et al [319]
Azotobacter sp., Mesorhizobium sp.	Siderophores	Ahmad et al., [14]
Mesorhizobium ciceri, Azotobacter chroococcum	Siderophores	Wani et al., [671]
Pseudomonas, Bacillus; Pseudomonas jessenii	Siderophores	Wani et al., [672]; Rajkumar and Freitas [493]

Bacillus sp. PSB10; Paenibacillus polymyxa	Siderophores	Wani et al., [671]; Ahemad and Kibret, 2014
Pseudomonas aeruginosa4EA; Enterobacter asburiae	Siderophores	Naik and Dubey, 2011; Ahemad and Khan, [9]

Lebin Thomas and Ishwar Singh (2019). Microbial Biofertilizers: Types and Applications Springer Nature Switzerland AG 2019 B. Giri et al. (eds.), Biofertilizers for Sustainable Agriculture and Environment, Soil Biology 55, https://doi.org/10.1007/978-3-030-18933-4_1.

Results and Discussion

Sustainable cassava agro-systems

The largest number of cassava diseases is found in Latin America and the Caribbean, the plant's centre of origin and sub-Saharan Africa and Asia according to FAO [181]. Many diseases are caused by pathogens, whose damage symptoms appear on the leaves, stems and storage roots [390]. The common diseases of cassava are cassava *mosaic* disease, cassava bacterial blight, cassava *anthracnose* disease, cassava bud *necrosis* and root rot. Some of these diseases attack the leaves and stems of cassava plants while others attack the storage roots, [438]. Cassava mosaic disease is caused by the African cassava *mosaic* virus which occurs inside the

leaves and stems and causes yield reductions of up to 90 percent [271]. The leaves become discoloured with patches of normal green colour mixed with light green, yellow and white area (*chlorosis*). When cassava mosaic attack is severe, the leaves become very small and distorted and the plants are stunted [323]. The symptoms are more pronounced on younger plants, usually under six months, than older plants. The disease is spread through infected cuttings and by whiteflies *Bemisia tabaci* [21]. China is world leading importer of cassava products, importing an estimated 9.5 million tons of flour and starch and also accounted for 63% world share of cassava import value in 2017 [182,430]. China ranked number one, exporting cassava worth of \$1.37billion while Canada's cassava export was \$57.8 million [430] (Table 3).

Table 3: Different microbial biofertilizers available in market and their application.

S. No.	Microbial biofertilizers	Trade names	Application
1	Azospirillum lipoferum, Azospirillum brasilense, and different strains of Azospirillum	Biospirillum, Green Plus, Bio-N, Azo-S, ROM, and	1) For normal and acidic soils and dry soils. (2) For paddy and other crops
2	Rhizobium, Azospirillum, Azotobacter, Bacillus sp., Acetobacter, Fungi sp.	ARATI BAJA liquid formulations *1	All crops
3	Enterobacter, Flavobacterium, Klebsiella, Mesorhizobium, Micrococcus, Fungi sp.	ARATI NAWOZ liquid formulations	All crops
4	Agrobacterium, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Enterobacter, Fungi sp.	OBD-Biofertilizer	All crops
5	Pseudomonas, Rhizobium, Rhodococcus, Bacillus, Fungi sp.	Gateway Biofertilizer *2	All crops
6	Azotobacter chroococcum, different strains of Azoto- bacter (non-symbiotic)	Bioazoto, Bhoomi Rakshak, Kisaan Azotobacter culture, and Azonik	For all crops like wheat, sorghum, barley, maize, paddy, mustard, sunflower, sesame, cotton, sugarcane, banana, grapes, papaya, water-melon, onion, potato, tomato, cauliflower, chilly, lady finger, rapeseed, linseed, tobacco, mulberry, coconut, spices, fruits, flowers, plantation, crops, and forest plants
7	Gluconacetobacter: diazotrophicus	Sugar-Plus	For sugarcane
8	Rhizobium strains (symbiotic, nitrogen fixing)	Biobium, Rhizo-Enrich, Kisaan Rhizobium culture, Rhizoteeka, Green Earth Reap N4, and Rhizonik	Pulses (gram, peas, lentil, moong, urd, cowpea, and arhar), oil legumes (groundnut and soyabeans), fodder legumes (barseem and lucerne), and forest tree legumes (subabul, shisam, and shinsh)
9	Phosphorus-solubilizing and Phosphorus-mobilizing microbes like Bacillus, megaterium, mycorrhizal fungi, etc.	Biophos, Get-Phos, MYCO-RISE, Kisaan P.S.B. culture, MycoRhiz, Reap P, and Phosphonive	For all crops
10	Potassium-mobilizing or potash bacteria like Bacillus mucilaginosus	BIO-NPK, Bharpur, BioPotash, Potash-Cure, and Green Earth Reap K	For all crops
11	Sulfur-solubilizing microbes like Thiobacillus thiooxidans	Biosulf, Sulf-cure, Sulphonik, S Sol B®, Siron, and MicroS-109	For cereals, millets, pulses, oilseeds, fiber crops, sugar crops, forage crops, plantation crops, vegetables, fruits spices, flowers, medicinal crops, aromatic crops, orchards, and ornamentals
12	Zinc-solubilizing microbes	Biozinc, Zinc-Cure, Zinc activator, Zinc extra and MicroZ-109	For crops like paddy, wheat, pulses, citrus, pomegranate, ginger, etc.
13	Silica-solubilizing microbes	BioSilica, Silica-Cure, and Silica-109	For crops like cereals, sugar cane, onions, leafy greens, legumes, cucumber, pumpkin, and gourd.

Visit website for material safety data sheet (MSDS)

*1 https://www.academia.edu/43310069/ARATI_BAJA_Liquid_Biofertilizer_Integrated_soil_fertility_management_ISFM_

*2 https://www.academia.edu/42632817/Gateway_Organic_Fertilizer_Biofertilizer_Gateway_Biofertilizer_Organic_3.0

Biofertilizer inoculant: Quorum sensing

The nitrogen-fixing efficiency of rhizobia bacteria, an important group of biofertilizers that contains organisms like *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium*, can vary till 450 kg N / ha among different strains and host legume species, in which root nodules are formed [232,574,575,614,615,631]. The rhizobial biofertilizers can be in powder, liquid, and granular formulations, with different sterilized carriers like peat, perlite, mineral soil, and charcoal [581]. In rhizobia, a nitrogen-fixing actinomycete, can form root nodules in several woody plants [68,139,151,266,607,663]. Other ecologically microbes include phosphorus-solubilizing bacteria (PSB) like *Bacillus* and *Pseudomonas* can increase phosphorus availability to plants by mobilizing it from the unavailable forms in the soil [503] called phosphorus-mobilizing biofertilizers or phosphate absorbers.

The mycorrhizal fungi form obligates or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is dependent on host for photosynthates and energy and in return provides a plethora of benefits to its host [567,593]. The mycelium of the fungus extends from host plant root surfaces into soil, thereby increasing the surface area for more efficient nutrient access and acquisition for the plant, especially from insoluble phosphorus sources and others like calcium, copper, zinc, etc. [563]. Mycorrhizal fungi enhance soil quality, soil aeration, water dynamics, and heavy metal and drought tolerance of plants and protects root pathogens or herbivores [505,593]. The inoculum source [75,301] determine the impacts on the results in amended and homogeneous crop growth.

Biofertilizer compost is produced from a wide variety of materials like straw, leaves, cattle-shed bedding, fruit and vegetable wastes, biogas plant slurry, agriculture waste, etc reported. Biofertilizer compost is biodegraded in anaerobic biodigester inoculated with broad spectrum decomposing microorganisms like *Trichoderma viridae*, *Aspergillus niger*, *A. terreus*, *Bacillus spp.*, several Gram-negative bacteria (*Pseudomonas*, *Serratia*, *Klebsiella*, and *Enterobacter*), etc. that have plant cell wall-degrading cellulolytic or lignolytic and other activities as narrated in Paper 1 [448]. Biofertilizer has proteolytic activity and antibiosis (by production of antibiotics) that suppresses other parasitic or pathogenic microorganisms [83].

The challenges of biofertilizer are lower shelf-life, temperature sensitivity, being contamination prone, and becoming less effective by low cell counts. Consequently, liquid formulations have been developed for *Rhizobium*, *Azospirillum*, *Azotobacter*, and *Acetobacter* which although costlier, have the advantages of having easier production, higher cell counts, longer shelf-life, no contamination, storage up to 45 °C, and greater competence in soil [419]. The application practices of microbial biofertilizers include seed treatment (plant growth regulator, PGR), seedling root dipping, and soil application. There are several microbial biofertilizers

available as dried or liquid cultures under different trade names in the market, which are used for a variety of purposes including enhancement of plant growth and soil fertility. For instance, the rhizobia biofertilizers can fix 50-300 kg N ha that increases yield by 10–35%, maintains soil fertility, and leaves residual nitrogen for succeeding crops [113,138]. The *Azotobacter* biofertilizer used for almost all crops can fix 20–40 mg N/g of carbon source that causes up to 15% increase in yield; maintains soil fertility; produces growth-promoting substances such as vitamin B complexes, indole acetic acid, and gibberellic acid; and is further helpful in biocontrol of plant diseases by suppressing some of the plant pathogens [2,321]. The phosphorus-solubilizing bacterial biofertilizers, which are nonspecific and suitable for all crops, produce enzymes which mineralize the insoluble organic phosphorus into a soluble form, thereby increasing crop yield by 10–30% [548,549,550].

Plant-microbe metagenomic

Biofertilizers mineralization in soil is an integral function of the microbe's inoculants with core mechanism for mineral phosphate solubilization is the production of organic acids and acid phosphatases. The mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms [379,551,556,644]. The rhizosphere makes a source of gene pool with a huge potential, particularly for agricultural applications with the aim to improve crop productivity and quality of agricultural products and shield crops from pests executed the genes responsible for PGPR activities and application of the recombinant molecules to soil.

An activity that improves the plant fitness and, hence, improves crop production is apparently ACC deaminase activity. Interestingly Nikolic et al., [426] also analyzed ACC deaminase genes (*acdS*) of bacterial endophytes colonizing field-grown potato plants and discovered the presence of two unique types of *acdS* genes, the dominant one showing high homology to an *acdS* gene derived from *P. fluorescens* through PCR analysis. The fundamental study on siderophores is mainly fascinating due to its triple function application, nutritional, systemic resistance inductor (ISR) and biocontrol reported by Bakker et al., [50]. The sequencing of whole genomes from a number of species permits to delineate their organization and provides the basis for understanding their functionality [392], as a consequence favoring metagenomic-agricultural practices.

Genomics contribution to agriculture spans the identification and manipulation of genes linked to specific phenotypic traits [706]. In addition to molecular breeding by marker-assisted selection of variants [344]. In the future, agricultural metagenomics without doubt aims to reveal several innovative solutions through the study of crops or livestock genomes, achieving information for protection and sustainable productivity for food industry reported by Bulgarelli et al., [89] and impacts on metagenomics study of the soybean rhizosphere [454]. Currently, the great majority of bacterial species are still unidentified [497]. Metagenomics application in

agriculture also proved to be suitable for depicting the multifarious patterns of interactions occurring among microorganisms in soil and in plant rhizosphere [454]; the environmental changes [454]; agricultural management [572] and can help decipher the role of soil microbes in plant nutrition [470] or in the cycle of elements [580].

Plant, soil, and microbiome also play a crucial role in agriculture provided that it determines plant fitness [244] and soil biogeochemical properties and affects both yield and quality traits [149]. Bio-nanotechnology applications which employ nanoparticles made of inorganic or organic materials could also provide new avenues for the development of carrier-based microbial *inocula* [360] like the cassava inoculants can be in biomaterials or nanomaterials called value added materials (VAS) will become carrier-based microbial *inocula* in the future climate-smart agriculture was reported by Otaiku et al., [448]; Otaiku et al., [449] posit that characterisation of advanced materials, and particularly of materials with necessarily complex structures such as bio and functional ones, requires analytical tools for observation and monitoring all relevant length scales (nano, micro, meso and macro).

Rhizosphere biocommunication

Rhizosphere microflora coordinated the expression of the specific target genes within the Rhizosphere and other eukaryotic cell types interactions of organisms to occupy particular habitat adapting to environmental conditions and against several adverse conditions. to share symbiotic and a symbiotic relationship with plants benefiting its growth directly or indirectly. These coordinated responses are generally induced by a group of chemicals signaling and are referred to as quorum sensing (QS) reported by Fuqua & Greenberg [201]. QS signals produced by rhizosphere microflora are significantly higher compared to other organisms isolated from

bulk soil. Scholars reports on QS signals with distinct chemical structures produced by Gram-negative rhizosphere bacteria and their potential to regulate a wide array of genes in the population [42,43,245,282,284,312,314,366,372].

Roots are reported to secrete exudates with a wide array of chemicals involved in regulation of both beneficial and pathogenic microorganisms in the rhizosphere. The variation in these chemicals helps in stabilizing the equilibrium of beneficial microbes in terms of its number and by which evading the continuous attack by soil borne pathogenic bacteria [47,662]. The cross talk between the plant roots and beneficial microbes is playing a vital role in growth and development of the entire plant kingdom in general and agricultural crops in particular [295,361,545]. The release of chemicals involved in trans-specific communication from roots can occur passively upon decay and may export signals actively to the extracellular environment. Ten to 40% of the photosynthetic carbon assimilation is released by the plants as root exudates, and it is a mixture of sugars, amino acids, organic acids, sugar alcohols, and secondary metabolites [46] microbial activity through nutrient bioavailability and regulating phytotoxic elements is vital [416,558]. The rhizosphere is a hotspot of several communications involving a wide range of microorganisms with diverse physiological importance [313,412,650]. Among these communications, quorum sensing and quorum quenching are attracting scientific community for its beneficial exploitation in plant growth regulations ultimately leading to yield enhancement. Gram-negative bacteria use homoserine lactones (LuxR/LuxI) as communication signals [532,587], whereas Gram-positive bacteria use oligopeptides in quorum sensing. During quorum sensing, it is important for the organisms to differentiate between species-specific signaling and signaling associated with interspecies behavior modulations [59,185,675].

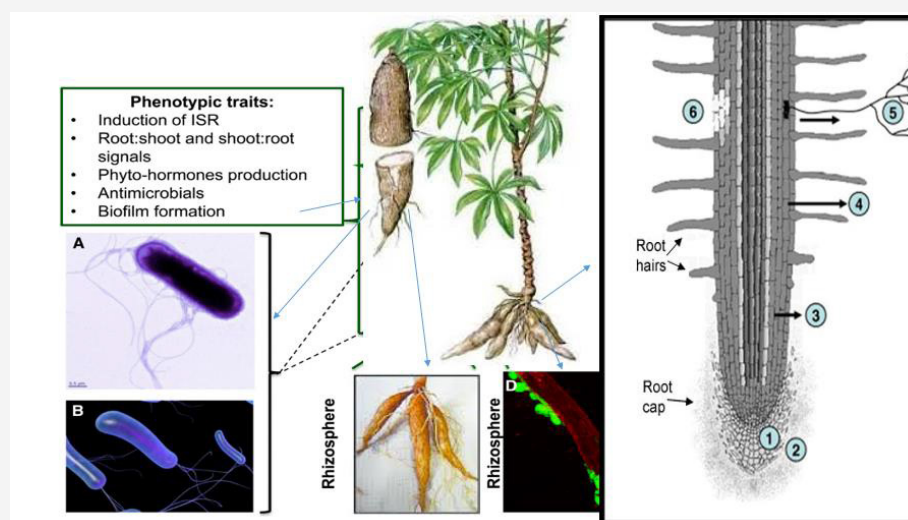


Figure 3: Schematic of a root showing the 6 major regions of rhizodeposits that may have occurred in the application of biofertilizer to cassava cultivation rhizosphere: carbon trading at the soil-root interface are. (1) loss of cap and border cells, (2) loss of insoluble mucilage, (3) loss of soluble root exudates, (4) loss of volatile organic carbon, (5) loss of C to symbionts, (6) loss of C due to death and lysis of root epidermal and cortical cells, adapted from Jones et al., 2009. Electron microscopic images of *B. subtilis* FB17 (A) and a single isolate of *Pseudomonas fluorescens* (B). B is from <http://www.buzzle.com/articles/pseudomonasfluorescens.html>, Appendix 2.

Mycorrhizal fungi support the growth of bacteria by releasing few nutrients, and in turn soil bacteria with its wide array of enzymes degrade the complex soil organic nutrients and make it easily available for the fungi [80,81]; supports the plant growth by extending its hyphae to the areas where plant roots are not able to reach; due to this extension, the plant gets sufficient nutrients supplied by both roots and fungi compared to uninfected roots. The law of limiting factor supported by adaptive radiation of the species is favored by coexistence of bacterial life with suitable interactions to scavenge the limiting factor required to colonize the specific habitat and suitably favored in competition with other groups of organisms [526,650]. Generally, quorum sensing in bacteria falls into three classes: the first is, as mentioned earlier, AHL-dependent LuxI/LuxR-type QS observed in Gram-negative bacteria, the second is the small peptide-mediated QS observed in Gram-positive bacteria, and the third observed in both these bacteria is luxS-encoded autoinducer 2 (AI²) QS. These signal molecules are operating with precise sensing and regulatory network [159,185,388,457,532,675]. Two types of quorum sensing systems are reported in Gram-positive bacteria in contrast to Gram-negative bacteria [159,191,194,427]. The first comprises of a three-component signaling peptide referred to as autoinducing peptide (AIP) and the other is a two-component signal transduction system which specifically responds to an AIP [159,388,532], Appendix 2 (Figure 3).

Biofertilizer impacts: Quorum sensing

Soil microorganisms in general and rhizosphere microflora in particular are considered as treasure houses of the soil defining its fertility and plant growth promotion. Table 4 represents QS signals and its regulated functions. Cell-to-cell signaling regulates the expression of the *rhlAB* operon responsible for production of biosurfactants [432,433,463,466]. *RhII*, N-butyryl homoserine lactone autoinducer synthase gene, and transcriptional activator encoding *rhlR* that are the major QS system and rhamnolytic transferase encoded by *rhlC* which is coordinately regulated

along with *rhlAB* are responsible for biosurfactant production in microorganisms [491]. These systems are under the influence of nutritional factor and QS signals [236]. They also came out with interesting observation that the nutritional conditions supersede cell-to-cell communication and hence correlate more positively with upregulation of quorum sensing-controlled genes such as *rhlAB*. Bacteria through social traits get several benefits such as coordinated population behavior (*Vibrio fischeri*, *Ps. aeruginosa*, and *Staph. aureus*), biofilm formation to get protection from adverse environmental conditions, nutrient and niche protection in nodules (*Rhizobium sp.*), enhanced colonization and growth in specialized niches (siderophores production for iron acquisition in bacteria), autolysis to provide nutrients and DNA for biofilm development (*Ps. aeruginosa*), coordinated movement toward nutrient source (*Yersinia sp.*, *Myxococcus xanthus*, *Ps. aeruginosa*), antibiotic resistance through production of extracellular enzymes to break down antimicrobials (*E. coli* and *Klebsiella spp.*), and also immune modulation to facilitate survival within the host (*Ps. aeruginosa*, *Porphyromonas gingivalis*, *Helicobacter pylori*) [150]. Microorganisms develop protective adaptations easily at high density than at low density to adverse environmental conditions such as acidic condition, alkalinity, pressure, etc. [131,195,196,336]. Usually, microorganisms develop protective adaptations easily at high density than at low density to adverse environmental conditions such as acidic condition, alkalinity, pressure, etc. [131,195,196,336]. Signaling pathways are well documented and described [339,418,425,473]. The link between soil signaling and nitrogen cycling is also investigated by De Angelis et al. [145]. They reported that many alpha-proteobacteria were newly found with QS-controlled extracellular enzyme activity, and even cell division, symbiotic plasmid transfer, gene expression in the rhizosphere, symbiosome development and nitrogen fixation, and nodule number in *Rhizobium* bacteria are regulated by QS. QS is also reported to play an important role in expression of genes associated with virulence factors [41,374,384,518,560,689] (Figure 4) (Table 4).

Table 4: Activities of soil borne bacterial functions regulated by Quorum Sensing (QS) signals [490].

S. No.	Taxonomic class	Genus or species	Cassava Inocula	QS signals produced	Known regulated functions	References
1	Actinobacteria	<i>Streptomyces sp.</i>	OBD-Biofertilizer	Gamma-butyrolactones	Antibiotic compound synthesis, differentiation	Chater [104] and Shaaban et al., [541]
2	Alpha- proteobacteri	<i>Agrobacterium tumefaciens</i>	OTAI AG®	OOHL	Ti plasmid transfer, virulence	White and Winans [681]; Otaiku et al., [448]
3	<i>Rhodopseudomonas palustris</i>		OTAI AG®	pCHL	Chemotaxis	Schaefer et al. [531]
4	<i>Mesorhizobium loti</i>		OBD-Biofertilizer	OHHL, OHL, DHL, ddHL	Nodulation	Yang et al., [692]; Otaiku et al., [448]
5	Gammaproteobacteria	<i>Pseudomonas aeruginosa</i>	OTAI AG®	BHL, OdDHL	Biofilm, elastase, lipase, alkaline protease,	Braeken et al., [85]
			OBD-Biofertilizer		HCN, pyocyanin, exotoxin A, swarming, lectins, rhamnolipids, virulence.	Ferluga et al., [187]; Otaiku et al., [448]

				PQS	Elastase, pyocyanin synthesis, LecA lectin, biofilm, AHL signaling, motility + intrinsic functions (antibiosis, iron chelation)	Dubern and Diggle, [156]
				DKPs (e.g., cyclo (D-Ala-LV al))	Unclear, cross-linked to AHL signaling	Holden et al., [258]
6		<i>Pseudomonas fluorescens</i>	OTAI AG®	OHHL, OHL	Biofilm formation, wheat rhizosphere colonization, biocontrol ability.	Wei and Zhang, [678]; Otaiku et al., [448]

BHL = N-butylryl-homoserine lactone | dDHL = N-dodecanoyl-homoserine lactone

DHL = N-decanoyl- homoserine lactone | OdDHL = 3-oxo-N-dodecanoyl-homoserine lactone

DKP = diketo-peptides | OHL = N-octanoyl-homoserine lactone | OOHL = 3-oxo-N- octanoyl-homoserine lactone

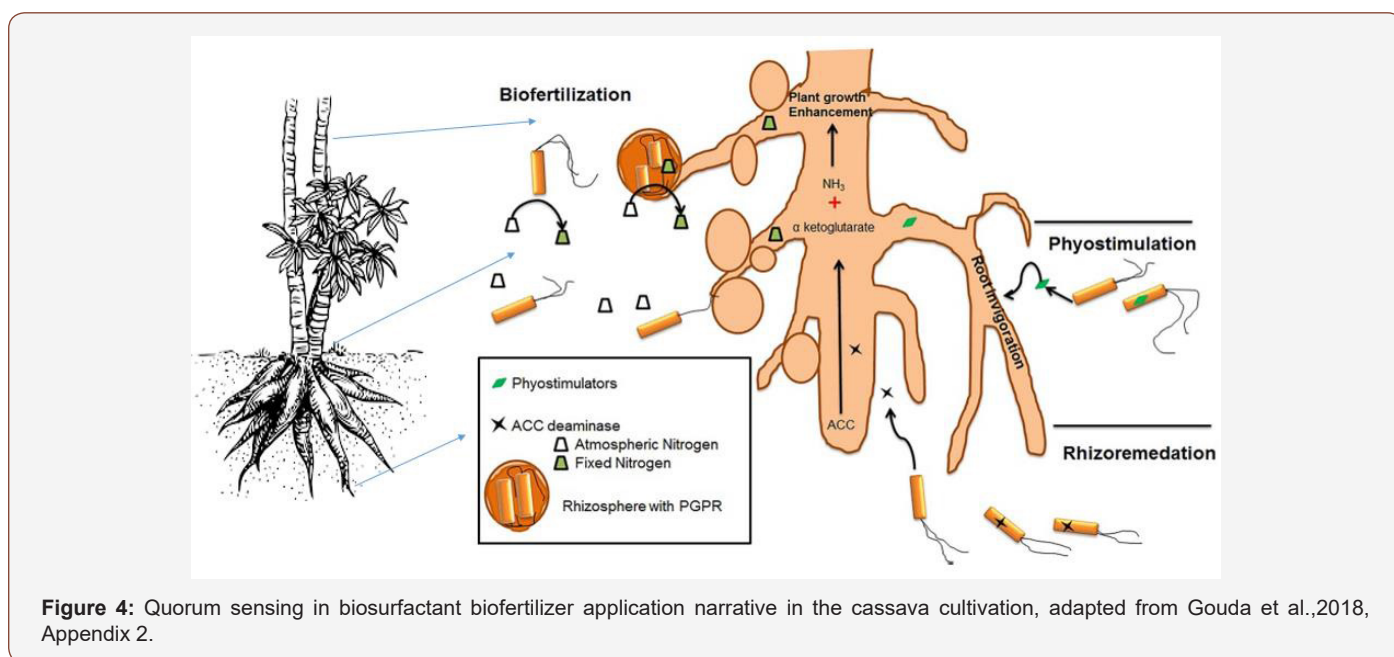


Figure 4: Quorum sensing in biosurfactant biofertilizer application narrative in the cassava cultivation, adapted from Gouda et al.,2018, Appendix 2.


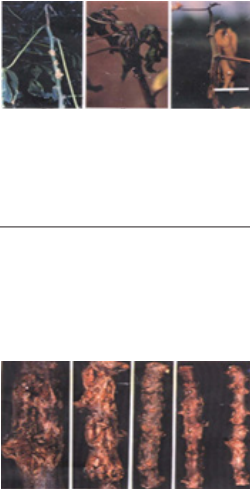
The plant microbiome has the ability to buffer plant hosts against abiotic and biotic stress, facilitate nutrient uptake and nutrient use efficiency, and promote growth [356,405,525]. Endophytic bacteria can be used to improve plant productivity and stress tolerance in the absence of pesticides and inorganic fertilizers, and to facilitate phytoremediation heavy metals and hydrocarbons, but more research is needed on how to best inoculate plants in field settings [93].

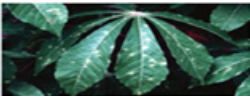


Diseases and pests of cassava



Cassava *anthracnose* disease is caused by a fungus which occurs on the surface of cassava stems and leaves [21] and appears as

cankers (sores) on the stems and bases of leaf petioles. Cankers weaken the petioles so that the leaf droops downwards and wilts [694]. The wilted leaves die and fall causing defoliation and shoot tip die-back or complete death of the shoot. Soft parts of cassava stems become twisted under severe attack by the disease. The disease usually starts at the beginning of the rains and worsens as the wet season progresses [323]. Cassava bacterial blight, Leaf spot diseases, Cassava brown streak disease, Cassava root rot diseases, cassava mealybug. The cassava green mite Table 4. The treatment of the cassava disease and pests by disease suppression bio-control broad spectrum microbial PGPR inoculants in the biofertilizer (Tables 5a,5b & 6) (Plates 1A & 1B).

Table 5: Impacts of Biofertilizer on Diseases and Pests of Cassava cultivation.

Diseases and Pests of Cassava								
The largest number of Cassava diseases is found in Sub-Saharan Africa, Latin America and the Caribbean								
N/S	Crops Components	Disease Infection	Pictures	PGPR Treatment	Biofertilizer	Impacts	References	
1	Leaves and stems	bacterial blight		Bacillibactin	Paenibacillus	Destroyed after root harvest	Alvarez et al., [21]; Singh et al., [562]	
				Diffusible metabolites as elicitors	P. polymyxa	yield losses of 20 to 100 %	IITA, [270]; Phi et al., [468]; Wen et al., [679]	
				Viruses		Fungicides and insecticides	FAO, [180]; Kumar et al., [318]	
	Integrated Nutrient Management			Giberellin (GA) production	OBD-Biofertilizer	Enhancing seed germination rate	Goswami et al., [230]	
				Insecticidal toxins	Bacillus, Pseudomonas,	Insecticidal Protein Production	PechyTarr et al. 2013; Roh et al., [508]	
				Pseudomonas and Bacillus species	PGPRs	Biocontrol agent called lipopeptide (LP) bio-surfactantsFacilitate root colonization	De Bruijn et al., 2007; Raaijmakers et al., 2010; Bais et al., [49]; Khabbaz et al., [297]	
2	Stems / bases of leaf petioles	Anthraxnose disease		Volatile organic compounds (VOCs)	PGPRs	Leaf droops downwards and wilts	Farag et al., [183]; Von Der Weid et al., [656]	
	A			Antimicrobials by Paenibacillus		Defoliation and shoot tip die-back	Yaninek et al., [694]	
				P. polymyxa		Microbial inhibiting agents	Abriouel et al., [5]; Phi et al., [468]	
				ISR against pathogenic bacteria		Indole acetic acid production	Lee et al., [332]; Lee et al., [333]	
	B					PGPRs rhizobacteriamediated ISR	Choudhary and Johri, [118]	
						Antifungal and antibacterial metabolites	Sessitsch et al., [539]; Sturz et al., [584]	
						Phosphate solubilisation	Wakelin et al., [661]	
						Supply of essential vitamins to plants	Pirttila et al., [472]	
	Integrated Nutrient Management					OBD-Biofertilizer	Microbes within the rhizosphere modify	
	Biofertilizer field application					PGPRs	modify root exudate composition Enhance growth	11ta 2008
							induce systemic resistance to subsequent pathogen attack	FAO, [181]
							Phytohormones used in plant defense, jasmonic acid, ethylene,	

3	Leaf	Leaf spot diseases				and salicylic acid, Infected leaves become yellow,	Kumar and Legg, 2009	
		White leaf spot, brown leaf spot, and leaf blight					dry and die prematurely Spots sometimes have purplish borders	FAO, [181]; IITA, [271]; Yaninek et al., [694]
	Integrated Nutrient Management			Pseudomonas antibiotics likes:	OBD-Biofertilizer	Bacterial ureases can control/kill the insect host	Salvadori et al., [522]; Nakkeeran et al., [404]	
				2,4-diacetylphloroglucinol (DAPG)	Pseudomonas genus	Produce antibiotics kill the growth of target pathogen	Glick et al., [224]; Santoyo et al., [525]	
				Pyoluteorin (Plt); pyrrolnitrin (Pm)	PGPRs	Antibiosis relies on the secretion of pathogenic molecules	Glick, [221,222]; Ahmad et al., [14]	
				phenazine-1-carboxylic acid (PCA)		Antibiotic has antifungal, antibacterial	Raaijmakers et al., 2002; Cronin et al., [129]	
				Protein-type (bacteriocins)		and antihelmintic properties	Loper and Gross, [342]; Velusamy et al., [645]	
				Hydrogen cyanide (HCN)			Thomashow and Weller [595]	
4	Leaf	Brown streak disease		Plant plasticity	PGPRs	Brown streak disease appears on the leaves	Miskito et al., [390]	
							stems and storage roots of cassava plants.	Goh et al., [226]
							Resistance to fungal pathogen infection	Chen et al, [112]
	Integrated Nutrient Management			Plant antioxidant system	PGPRs Endophyte	Degrade organic pollutants	Viñaset al., 2005, Taghavi et al., [591]	
					Pseudomonas	Secondary metabolites for plant defense and communication	Kirby and Keasling, [299]	
5	Root	Root rot diseases		Exopolysaccharides (EPS)	PGPRs	leaves on cassava plants affected by root rot disease turn brown,	IITA, [271]; FAO, [181]	
					lipopolysaccharide-proteins		Defence for the root tip	Curlango-Rivera et al., [130]
					Polysaccharide-lipids		Protective biofilm on the root surface	Zahran, [700]; Morris and Djordjevic, [393]
					Plant root architecture		Wilt and the plant appear scorched.	Olugbenga et al., [438]
						Post-harvest control	Allard et al., [19]	
	Integrated Nutrient Management			Siderophore Production	OBD-Biofertilizer	Catalyst in enzymatic processes, oxygen metabolism,	Aguado-Santacruz et al., [7]	
				Plasticity in a given environment	PGPRs	electron transfer and DNA and RNA syntheses	Friesen et al., 2011, Spaepen, et al., [573]	
				Rhizosphere microbiome		Function in root disease suppression	Martinez-Viveros et al. [364]; Neilands [415]	
			Plant root architecture		Competitive fitness of plant growth-promoting	Barton and Abadia [58]; Weber, [677]		

				Phytobiome / Microbiome		Sequester iron and forms complexes with other essential elements,	Bellenger et al., [64]; Braud et al., 2009a, b; Albrecht-Gary and Crumbliss [16]; Burdon and Thrall, [92]
6	Pests: Stems and leaves	Mealybug		Insect herbivores		Reduces the lengths of the internodes and causes the leaves	Pieterse et al., [469]
		Phenacoccus manihoti		Fungi		to clump together into 'bunchy tops'	Mavrodi et al., 2011; Sang et al., [524]
						Reduce leaf and root yield, sometimes by as much as 80 %	Antonopoulos et al., [27]
						Yield loss of 60 percent of the roots	Tjamos et al., [604]; Gkizi et al., [219]
						100 percent of the leaves.	
	Integrated Nutrient Management			Hydrogen Cyanide Production	OBD- Biofertilizer	biocontrol of certain plant pathogens	Martinez-Viveros et al., [364]; Gupta et al., [238]
				hcnAB genes detecting	PGPRs	Pseudomonas strains producing antibiotic 2,4-DAPG	Haas and Defago [240]; Svercel et al., [586]
				HCN-producing pseudomonas		HCN is also involved in metal sequestration	Wongfun et al. [682]; Rijavec and Lapanje [504]
				Bacterial volatile compounds (BVCs)		Trigger plant growth and immunity	Chung et al., [119], Mavrodi et al., 2011
				Pathogen suppression		Does not require any established physical contact	Ortiz-Castro et al. 2009
				Paenibacillus kill larvae of pest insects		to trigger growth response. (Paenibacillus).	Sharma et al., [548]; Neung et al., [417]
						BVCs such as 2,3-butanediol and acetoin accelerate plant growth and induce systemic resistance	Audrain et al., [34]; Ryu et al., [516]
						BVC which strikes the plant's physiology and defense	D'Alessandro et al., [133]
				Trait plant hormone signaling		Use natural enemies such as predators, parasitoids	Jang et al., [281]
					and parasites e.g .ladybirds.	FAO, [181]	
7A	Pests: Green mite (Leaves)	Mononychellus tanajoa				Severe mite attack can result in 13 to 80 % loss in cassava yield	Alvarez et al., [21]
						Lowland areas with a prolonged dry season.	Olugbenga et al., [438]



						Heavily attacked leaves become stunted and deformed.		
						Pest causes tiny yellow chlorotic leaf spots, the size of pin pricks, on the upper leaf surfaces.		
	Integrated Nutrient Management			Trait plant hormone signaling	OBD-Biofertilizer PGPRs	Plant systemic resistance	Mendes et al., [386]; Hu et al., 2003	
7B	Pests: White flies (Leaves)	Aleurodicus dispersus						
		Spiraling white flies				Herbivory, and defense	Friesen et al., 2011	
						Damage cassava by sucking sap from the leaves.	Paterson et al., [458]	
						They secrete large amounts of honeydew that supports		
						the growth of black sooty mould on the plant, causing premature fall of older leaves.	Alvarez et al., [21]	
		Integrated Nutrient Management			ACC deaminase	OBD-Biofertilizer	Variety of stochastic disturbances	Baig et al., [45]
						PGPRs	Microbiome mediated physiology	Van Kleunen, et al., [630]
8	Termites (Chew and eat stem cuttings)			Nematodes			Khan et al., [298]	
	Cassava root scale	Stictococcus vayssierrei				Causing the cassava to grow poorly and die or rot.		
	Cassava white scale	Aonidomytil usalbus				Cause the tubers to be smaller and deformed, and stems to lose a lot of water and die		
	Integrated Nutrient Management			"induced systemic tolerance (1ST)"	OBD-Biofertilizer	Enhance survivability of stress-affected plants	Yang et al. [692]	
					Bacillus subtilis	PGPRs	modulation of hormonal status in host plant	
					Fluorescent pseudomonads		Increase in stress ethylene level plant In response to stress stimuli of (salinity, drought, metal toxicity, etc.	Singh and Jha, [565]
						VOC produced by Bacillus subtilis confers salt tolerance reduce the growth inhibitory effects of stressors	Zhang et al., [638]; Penrose and Glick, [462]	

Table 5B: Annotation of the pictures of Bio-control impacts Biofertilizer Cassava crops.

N/S	Picture Description	Infection Cause	References
1	Water soaked lesions on plant leaves	Bacterium, fungus Colletotrichum gloeosporioides	Legg, [324]; Thresh et al., [597]
		gloeosporioides f.sp	Tiendrebeogo et al., 2012
2	Cassava Anthracnose disease shoot symptom Infection cause		Fokunang et al., [177] Saunders et al., [519]
A.	P1 = Young cassava plant with shoot die-back symptoms	Cankers on stems, leaf spot and tip die back.	Mulenga et al., [401]; Ikotun, [272]
	P2= Cassava seedling with shoot die-back and wilt symptoms		Legg, [322]
	P3 = Seedlings. arrow shows point of infected leaf defoliation		Legg, [324]
B.	P1 = Large deep expanding cankers	Numerous small circular sunken spots on the leaf lamina	Mabasa, [351]
	P2 Crocodile-like deep anthracnose cankers		Legg and Raya [326]
	P3 = superficial invading cankers	Petiole infection by hyphae growing from lamina	Legg et al., [325]
	P4 = Nonexpanding superficial cankers		CABI [694]; Ikotun, [273]
3	Plant with white leaf spot symptom	Fungi spread to new plants from these sources by wind or rain splashes	IITA, [271]; FAO, [181]
4	Brown streak disease	Plant with brown leaf spots	Ikotun, [273]
		Leaves, the disease appears as patches of yellow areas mixed with normal green color.	FAO, [181]
			IITA, [271]
5	Cassava root rot diseases	fungi living on the root or in the soil	Miskito et al., [390]
6	a. Cassava mealybugs	Mutation in somatic cells	FAO, [181]; Vanderschuren et al., [638]
	b. Leaf distortion caused by cassava mealybugs	Pests	IITA, [271]
7A	a. Cassava green mite	Pests	Thresh et al., [599]
	b. Speckled appearance on cassava leaves caused by cassava mites.		Mahungu et al., [352]
	a. Adult whiteflies	Pests	Legg, [323]
7B	b. Whiteflies feeding on the underside of cassava leaf	Pests	Thresh and Cooter, [598]
	c. Chlorosis and sooty mould on cassava leaves as a result of whiteflies feeding.	Pests	Legg, [323]; James et al., 2000

Table 6: List of some beneficial plant growth-promoting traits in the OBD-Biofertilizer.

Traits	Role	Microbe	References
Phosphate solubilization	1. Organic acid production	Bacillus licheniformis; B. amyloliquefaciens; Penicillium sp.	Chen et al., [113] and Wakelin et al., [661]
	2. Phytase production	Bacillus mucilaginosus; Aspergillus niger	Vassilev et al. [642]; Ryu et al. [516]
	3. Phosphatase production	Serratia marcescens	Ryu et al. [517] and Unno et al., [616]
Nitrogen fixation	1. Symbiotic	Vesicular-arbuscular mycorrhizal fungi	Shah et al., 2010
	2. Non-symbiotic	Gluconacetobacter diazotrophicus	Bhattacharyya and Jha, [74]
Phytohormone production	1. IAA production	Bacillus licheniformis; Penicillium sp.	Goswami et al., [230] and Waqas et al., [673]
	2. Cytokinin production	Bacillus megaterium	Castro et al., [99]
	3. Gibberellin production	Acetobacter diazotrophicus, Penicillium sp.	Basti et al., [60] and Waqas et al., [673]
Biocontrol	1. Extracellular enzyme production		
	(a) Chitinase	Enterobacter agglomerans	Nielsen and Sorensen [420]
	(b) Glucanase	Bacillus cepacia	Compant et al., [124]
	2. Antibiotic production	Pseudomonas fluorescens; Trichoderma koningii	Thomashow and Weller, [595]; Xiao-Yan et al., 2006

	3. Siderophore production	<i>Pseudomonas aeruginosa</i>	Braud et al., 2009a, b
	4. HCN production Production	<i>Pseudomonas chlororaphis</i>	Nandi et al., [406]
Potassium solubilization	Production and excretion of organic acid and inorganic acid	<i>Bacillus mucilaginosus</i>	Ullman et al., [613]
Induced systemic tolerance	1. ACC deaminase production	<i>Trichoderma</i> , <i>Asperellum</i> ; <i>Penicillium citrinum</i>	Mayak et al., [369]; Viterbo et al., [655]; Jia et al., [285]
	2. Exopolysaccharide production	<i>Oceanobacillus</i>	
	3. VOC production	<i>Bacillus amyloliquefaciens</i>	

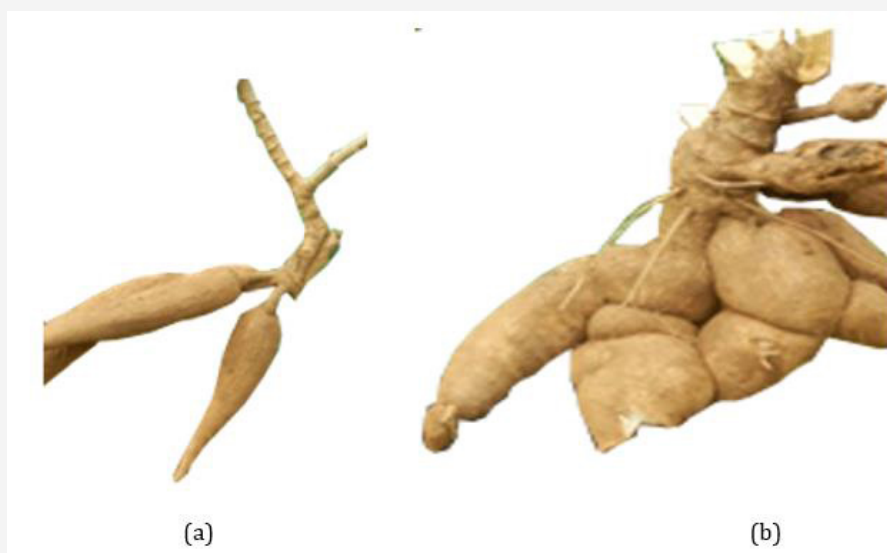


Plate 1: (a)World cassava average yield 12.2 t/ha; (b) Biofertilizer treated yield 25-32 tons/ha [448].

Microorganisms affecting stress tolerance

Bacteria with the potential to act as bio stimulants have been isolated from a number of ecosystems with saline, alkaline, acidic, and arid soils. These bacteria belong to several genera such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Bacillus*. Members of these genera have developed strategies to adapt and thrive under adverse conditions [40,389]. Amongst these adaptations, alterations to the composition of the

cell wall and the ability to accumulate high concentrations of soluble solutes are common. These allow for enhanced water retention and increased tolerance to osmotic and ionic stress. Cell wall composition is altered through enrichment for exopolysaccharides (EPS) and lipopolysaccharide-proteins and polysaccharide - lipids which form a protective biofilm on the root surface [235,700]. Plant growth-promoting rhizobacteria (PGPR) inoculated soils can ameliorate plant abiotic stress responses (Tables 7&8).

Table 7: Progress and current status of cassava genetic transformation.

N/S	Explant	Regeneration mode	Gene-transfer technique	Plasmid marker genes	Selection	Target traits	Integration/ expression	References
1	Somatic cotyledon	SE	Agrobacterium	pGV1040(nptII,bar,uidA)	ppt	Herbicide resistance	SAP, GAP	Sarría et al. 2000
2	Suspension	SE	Agrobacterium	pHMG(uidAint,hpt,pmi)	Hygromycin, mannose	Herbicide resistance	SAP, NAP, GAP	Zhang et al. 2000b
3	Suspension	SE	Agrobacterium	pCP15GUS; pCP54GUS (uid Aint,hpt)	Hygromycin,	Root-specific promoters	SAP, NAP, GAP	Zhang et al. 2003b
4	Suspension	SE	Agrobacterium	pCASP1(uidAint,hpt)	Hygromycin	Improved protein content	SAP, NAP, GAP, WAP	Zhang et al. 2003a
5	Suspension	SE	Agrobacterium	patatin-CYP79D1/D2(nptII)	Paromomycin	Reduced cyanogen content	SAP, RAP	Siritunga and Sayre 2004
6	Suspension	SE	Agrobacterium	pILTAB9001(nptII)	Paromomycin	CMD resistance	SAP, NAP	Chellappan et al. 2004
7	Somatic cotyledon	SE	Agrobacterium	3D(nptII)	Paromomycin	improved starch content	SAP, RAP	Ihemere et al. [278]
8	Somatic cotyledon	SE	Agrobacterium	pMAT21; pEXM2; pIPT5(ipt)	Kanamycin	marker-free	SAP, RAP, GAP, GAP	Saelim et al. 2009

9	Suspension	SO	Agrobacterium	pCP2	Hygromycin	tissue-specific promoter	SAP, GAP	Beltr' an et al. 2010
10	Suspension	SE	Agrobacterium	pSG529(nptII)	Paromomycin	prolonged leaf life	SAP, RAP	Zhang et al. [710]
11	Suspension	SE	Agrobacterium	pILTAB600; pILTAB601(nptII)	Paromomycin	mproved protein content	SAP, WAP	Abhary et al. [710]
12	Suspension	SE	Agrobacterium	p35S: GBSI-RNAi; p54/1.0	Ygromycin	waxy cassava	SAP, RAP,	Zhao et al. [679]
13	Suspension	SE	Agrobacterium	RNAiFL-CP (nptII)	Paromomycin	CBSVDresistance	SAP, NAP	Yadav et al. [688]

Adapted from Liu et al., [338], AGPase, ADP-glucose pyrophosphorylase; bar, bialaphos resistance gene; GAP, beta-glucuronidase analysis positive; hpt, hygromycin phosphotransferase gene; ipt, isopentenyl transferase gene; luc, luciferase gene; NAP, Northern analysis positive; nptII, neomycin phosphotransferase II gene; pat, phosphinothricin acetyl transferase gene; pmi, phosphomannose isomerase gene; RAP, reverse transcription-polymerase chain reaction analysis positive; SAP, Southern analysis positive; SE, somatic embryogenesis; SO, shoot organogenesis; TGE, transient gene expression; uidA, beta-D-glucuronidase gene; uidAint, uidA with intron; WAP, Western analysis positive.

Table 8: Microbial Biofertilizers: Market Types and Application.

S. No.	Microbial biofertilizers	Trade names	Application
1	Azospirillum lipoferum, Azospirillum brasilense, and different strains of Azospirillum	Biospirillum, Green Plus, Bio-N, Azo-S, ROM, and	1) For normal and acidic soils and dry soils. (2) For paddy and other crops
2	Rhizobium, Azospirillum, Azotobacter, Bacillus sp., Acetobacter, Fungi sp.	ARATI BAJA liquid formulations *1	All crops
3	Enterobacter, Flavobacterium, Klebsiella, Mesorhizobium, Micrococcus, Fungi sp.	ARATI NAWOZ liquid formulations	All crops
4	Agrobacterium, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Enterobacter, Fungi sp.	OBD-Biofertilizer	All crops
5	Pseudomonas, Rhizobium, Rhodococcus, Bacillus, Fungi sp.	Gateway Biofertilizer *2	All crops
6	Azotobacter chroococcum, different strains of Azotobacter (non-symbiotic)	Bioazoto, Bhoomi Rakshak, Kisaan Azotobacter culture, and Azonik	For all crops like wheat, sorghum, barley, maize, paddy, mustard, sunflower, sesamum, cotton, sugarcane, banana, grapes, papaya, water-melon, onion, potato, tomato, cauliflower, chilly, lady finger, rapeseed, linseed, tobacco, mulberry, coconut, spices, fruits, flowers, plantation, crops, and forest plants
7	Gluconacetobacter: diazotropicus	Sugar-Plus	For sugarcane
8	Rhizobium strains (symbiotic, nitrogen fixing)	Biobium, Rhizo-Enrich, Kisaan Rhizobium culture, Rhizoteeka, Green Earth Reap N4, and Rhizonik	Pulses (gram, peas, lentil, moong, urd, cowpea, and arhar), oil legumes (groundnut and soybeans), fodder legumes (barseem and lucerne), and forest tree legumes (subabul, shisam, and shinsh)
9	Phosphorus-solubilizing and Phosphorus-mobilizing microbes like Bacillus, megaterium, mycorrhizal fungi, etc.	Biophos, Get-Phos, MYCO-RISE, Kisaan P.S.B. culture, MycoRhiz, Reap P, and Phosphonive	For all crops
10	Potassium-mobilizing or potash bacteria like Bacillus mucilagenosus	BIO-NPK, Bharpur, BioPotash, Potash-Cure, and Green Earth Reap K	For all crops
11	Sulfur-solubilizing microbes like Thiobacillus thiooxidans	Biosulf, Sulf-cure, Sulphonik, S Sol B®, Siron, and MicroS-109	For cereals, millets, pulses, oilseeds, fiber crops, sugar crops, forage crops, plantation crops, vegetables, fruits, spices, flowers, medicinal crops, aromatic crops, orchards, and ornamentals
12	Zinc-solubilizing microbes	Biozinc, Zinc-Cure, Zinc activator, Zinc extra and MicroZ-109	For crops like paddy, wheat, pulses, citrus, pomegranate, ginger, etc.
13	Silica-solubilizing microbes	BioSilica, Silica-Cure, and Silica-109	For crops like cereals, sugar cane, onions, leafy greens, legumes, cucumber, pumpkin, and gourd

Visit website for material safety data sheet (MSDS)

*1https://www.academia.edu/43310069/ARATI_BAJA_-Liquid_Biofertilizer_Integrated_soil_fertility_management_ISFM_

*2https://www.academia.edu/42632817/Gateway_Organic_Fertilizer_Biofertilizer_Gateway_Biofertilizer_Organic_3

Endophytes are microorganisms that live within the plants' tissues without causing any damage to the host. Endophytes could be classified as fungi, bacteria or algae [535]. Endophytes primarily

assist in promoting the growth of plants that they inhabited as shown Figure 2 and Table 1. Facultative endophytes grow outside its host plant, obligate endophytes are dependent on their host plant

for their growth and survival. Endophytic bacteria are correlated with the enhanced plant growth by the production of hormones that increase accessibility of nutrients, such as nitrogen, potassium and phosphorus reported by Glick, 2012, Table 8 While induced disease resistance activities are allied with the abilities to produce secondary metabolites, such as antibiotics or chitinase enzyme, which can inhibit growth of plant pathogens and act as biocontrol agents [128,670].

Endophytic bacteria have the capacity to cope with phytopathogenic fungi with induced systemic resistance (ISR) [469]. Due to their beneficial function such as plant growth promotion and disease control, endophytes can be used in the form of bio-formulations (seed treatment, soil application and seedling dip) in agriculture. Endophytic bacteria can also induce seedling emergence and stimulate plant growth under stress conditions. The genera of *Bacillus* and *Pseudomonas* are identified as frequently occurring bacteria in agricultural crops. It has been reported that most of Gram-negative endophytes act as agents of biological control [302], while among the Gram-positive bacteria, the dominant endophytic species are *Bacillus* species. The root exudates contain that colonize different bacterial genera and they differ normally according to plant species. The apical root zone having thin-walled surface of root cells includes cell elongation and the root hair zone (zone of active penetration), and the basal root zone with small cracks are the preferable sites of bacterial attachment and subsequent entry caused by the emergence of lateral roots (zone of passive penetration) of lateral roots (penetration). Root colonization or rhizospheric beneficial microorganisms are familiar biocontrol agents and plant growth promoters. Innumerable compounds such as hydrocyanic acids (HCN), DAPG, phenazines, pyrrolnitrin, enzymes and phytohormones to protect plant from toxic effect of fungal pathogens are considered as the significant products to help endophytes to be colonized in rhizosphere, Figure 4. Bacteria are able to trigger signaling pathways to produce extracellular metabolites with higher toxicity for other microorganism lead to destruction of higher pathogen, called induced systemic resistance (ISR).

Myriad of bacteria has been documented for beneficial effects, alleviation of several abiotic and biotic stresses. *Pseudomonas* and *Bacillus* sp. have been studied as potential candidate to provide ISR to plants. On an average, most of mineral nutrients in soil are present in millimolar amounts but P is present in micromolar or even lesser quantities. However, plants are well adapted to uptake of P from low concentration soil solution. Therefore, it is presumed that the supply and availability of P to the root surface is influenced by the root and microbial processes. Schematic illustration of important mechanisms known for plant growth promotion by PGPR. Different mechanisms can be broadly studied under (1) Biofertilization, and (2) Biocontrol of pathogens. Biofertilization encompasses: (a) N₂ Fixation, (b) Siderophore production, (c) P inorganic solubilization by rhizobacteria. Biocontrol involves:

(a) Antibiosis, (b) Secretion of lytic enzymes, and (c) Induction of Systemic Resistance (ISR) of host plant by PGPR.

Growth, Yield and Root Quality: Biofertilizer

Biofertilizer facilitate the below-ground biological activity of earthworms, bacteria and fungi, and supply a wide range of nutrients, including secondary and micro-nutrients Adoa reported highest plant height with the application of poultry manure on Nkabom and IFAD cassava varieties. Adjei-Nsiah & Issaka (2013) observed that average fresh tuber yield increase from 13.7 t/ha without amendment to 23.7 t/ha with application of 4 t/ha poultry manure and compared with where biofertilizer application at 5t/ha yield ; 16 t/ha and control yield 12 t/ha [448], Plates 1 and 2 Organic fertilizer promote the growth of stems and leaves of cassava, increase the chlorophyll content and the photosynthesis of leaves and improve the physiological metabolism of cassava. The period of maximum rate of dry matter partitioning depends on genotype-by-environmental interaction. Canopy spread in cassava ensures large surface solar interception for photosynthesis. Nutrient supplied by poultry manure enhances increase in plant height due to increase in cell elongation of plant tissues as a result of steady release and mineralization of nutrients.

Amanullah et al., (2006), Parkes et al., (2012) observed that the number of roots per plant was significantly influenced by organic fertilizer treatment steady availability of nutrients throughout the crop growth period favourable changes in soil, such as loose and friable soil conditions, enabling better root formation, Plate 1. An increase in the number of storage roots per plant in response to organic fertilizer application has been reported by Kasele (1980) and Pellet & El- Sharkawy (1997). Leo & Kabambe (2014), observed a significant increase in number of roots per plant, and tuber diameter having a positive correlation with fertilizer treatment [448]. Manure application has resulted in higher root yields of cassava. Manure application enhances the cooking quality (mealiness) of cassava. Various observations have been made of a positive correlation between dry matter content and cooking quality of cassava.

Findings

Biofertilizer biodegradation

Metals are directly and/or indirectly involved in all aspects of microbial growth, metabolism and differentiation [205]. Bacterial resistance mechanisms generally involve efflux or enzymic detoxification, which can also result in release from cells, e.g. Hg (II) reduction to Hg (0) [421-424,450,510,553,554]. Bacterial plasmids have resistance genes to many toxic metals and metalloids, e.g. Ag⁺, AsO₃, Cd²⁺, CrO₂, Cu²⁺, Hg²⁺, Ni²⁺, Sb³⁺, TeO₂ and Zn²⁺. Related systems are also frequently located on bacterial chromosomes, e.g. Hg²⁺ resistance in *Bacillus*, Cd²⁺ efflux. *Bacillus* and arsenic efflux in *Escherichia coli* [443,445,510,553]. Microbes are intimately associated with the biogeochemical cycling of

metals, and associated elements, where their activities can result in mobilization and immobilization depending on the mechanism involved and the microenvironment where the organism (s) are located [165,210,211,654].

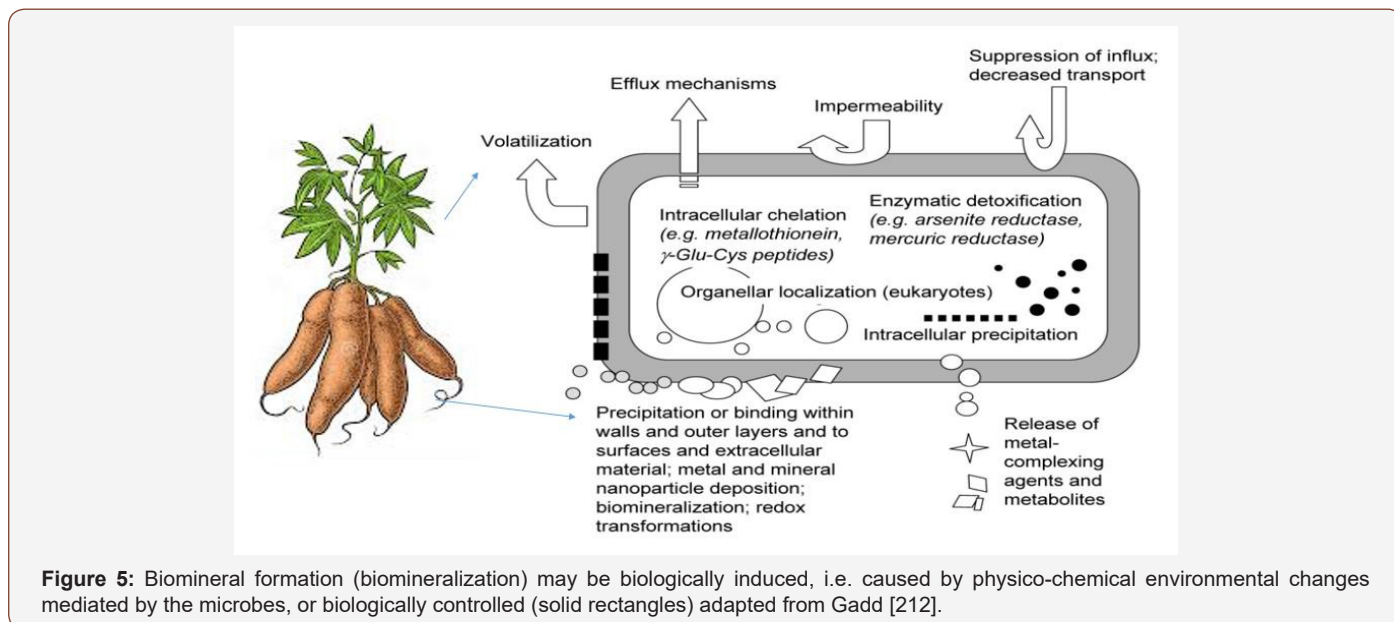
Despite apparent toxicity, many microbes grow and even flourish in apparently metal-polluted locations, and a variety of mechanisms, both active and incidental, contribute to resistance [35,193,203,259,397] Biofertilizer can be used for biodegradation of hydrocarbon pollution soil that impacts Niger Delta cassava cultivation (Gure, Yorla and Kpean) during hydrocarbon exploration reported by Otaiku, 2019 where total N for the impacted soils range (0.58 to 0.179%). The concentration range for N% was <4.5 for deficient reported by Howeler [263]. The available P of the impacted soils ranges from 0.011 to 0.019%. The critical concentration for deficiency is $P < 0.2\%$ for cassava growth [263]. The polluted soil can be remediated and fortified with biofertilizer OBD-Biofertilizer inoculated with OTAI AG ® microbes to support cassava growth reported by Otaiku et al., [448].

Microbial resistance to toxic metals is widespread, with frequencies ranging from a few per cent in pristine environments to nearly 100 % in heavily polluted environments [554]. Chemical and biological methylation BY microbes playing significant roles in the latter process [208,594]. All microbial materials can be effective bio sorbents for metals except for mobile alkali metal cations like Na^+ and K^+ , and this can be an important passive process in living and dead organisms [207,209,582,669]. Microbial biodegradation of organometallic (and organometalloid) compounds, still widely used in agriculture and industry, can result from direct enzymic action, or by microbial facilitation of abiotic degradation, e.g.

by alteration of pH and excretion of metabolites [208,209] other xenobiotic that may be anthropogenically produced like TNT, RDX and other heavy metals [165,345,442-445]. The insoluble glycoprotein glomalin, produced in copious amounts on hyphae of arbuscular mycorrhizal fungi, can sequester such metals, and could be considered a useful stabilization agent in remediation of polluted soils [229]. Phytostabilization strategies may be suitable to reduce the dispersion of uranium (U) and the environmental risks of U-contaminated soils. Biomineralization is itself an important interdisciplinary research area, and one that overlaps with geomicrobiology [51,52,153,304]. There is growing awareness of the geochemical significance of microbes among researchers in geology, mineralogy, geochemistry and related disciplines [2 3,51,82,212,220,304,354,618,674]. Xenobiotic chemicals which may be carcinogens [444], drugs, food additives, hydrocarbons, pesticides, and many other forms of environmental pollutants. The chemical reactions in soil necessarily should facilitate conversion of xenobiotic to simpler compounds (mineralization) or sometimes alternatively xenobiotic undergo activation (conversion into toxic molecule). almost all the organic compound can be mineralized under this process [362]. Munition xenobiotic can be biodegraded by bacteria [443,445] and Fungi [442].

The Rhizosphere engineering: rhizomicrobiome for better plant health

In a rhizosphere microbiome, not all of the microbes are needed to fulfill the ecological services to plants because functional redundancy in microbial communities across diverse environments is common [152,572] (Figure 5).



The plants and the associated microbes are not seen individually as a unit of inheritance and evolution, rather as a holobiont or superorganism. The approach involves microbial population engineering rather than single strain engineering. The rhizosphere engineering holds great promise for future plant breeding programs

and biotechnological application like in cassava crop. Microbiome assembly can be very sensitive to host genetic and environmental parameters and can vary even between different plant tissues. Rhizosphere microbiome diversity and their inheritance had been projected to be equally important as that of plant genome, since

number of genes in plant microbiome is more than number of genes in a host [371]. The rhizosphere management methods should primarily focus on the hypothesis of increase in yield by altering the dynamics of host genotype-x-environment-x-microbe interactions [93] like in cassava crop and the ability to manage and manipulate microbiome is limited. There are three main approaches in building a productive microbiome - the first one relies on construction of a high yielding microbial consortium, second and third approaches involve manipulating the plant or the superorganism respectively.

Rhizosphere environment variations are induced by altering the physical and chemical environment in the rhizosphere through plant- affected characters which change the spectrum of the fitness and interactions among microbes and evolution of new microbes better suited to the rhizosphere environment [329]. These changes in microbiome structure and function are usually attributed to differences in root exudate chemistry [46,48,498], root architecture and in plant nutrient uptake rates [62] which makes it possible to engineer these traits into crops through gene editing tools. The most direct way to alter the microbiome is through inoculation with several strains or mixed cultures of bacteria, fungi rhizobia, endophytes etc. designated as biofertilizers. The concept of synthetic microbial consortium (SMC) is different from co-cultures, mixed cultures, microbial consortia and other similar concepts in a way that it includes, not only living together but also labor division [141,234,511,623].

There are two ways for designing and constructing SMCs [288].

The Meta-organism or superorganism approach is based on the fact that both microbiome and the plants are highly dependent on each other as the microbiome contributes a significant portion of the secondary genome of the host plant. The heritability of the meta organisms is not solely dependent on the genetics of microbes but the genetics of host plant as well. No general-purpose framework for the reconstruction of SMCs used to promote plant health is yet available [93]. The existence of functional redundancy in microbial communities across diverse environments is common [152,572]. Based on relative occurrence of microbes in microbiomes can be classified as core or minimal microbiome. A core microbiome (CM) is comprised of the members common to two or more microbial assemblages associated with a habitat [243,612].

There are various ways to define the CM within a habitat using bioinformatics-based approaches. Shade and Handelsman [542,543] suggested five parameters, including membership, composition, phylogeny, persistence, and connectivity, to discover the core microbiota based on a Venn diagram analysis. The concept of minimal microbiome (MM) implied the smallest but functionally indispensable subset of the total microbiome [482]. The ultimate goal of identifying such CMs or MMs is to exploit them in reconstruction of synthetic microbial consortium (SMC) with desirable member microbes [241]. SMCs are composed of multiple species with well-defined genetic background and help in accomplishing specific function through interactions among

microorganisms. Plants release 10-20% of their photosynthates as exudates, which alter the physical and chemical properties of soil that in turn provides suitable niches for microbial proliferation [161,698]. Root exudates include a wide range of compounds, like carbohydrates, amino acids, organic acids, fatty acids, nucleotides, flavones, vitamins, and enzymes [54].

Cassava inoculants: Biofertilizer

Plant growth-promoting bacteria (PGPB) are generally obtained from soils [123,128]. *Bacillus species including Lysinibacillus sphaericus, B. amyloliquefaciens B. cereus, B. mycoides, B. subtilis, B. pasteurii, B. pumilus, and B. thuringiensis* may reduce the incidence or severity of plant diseases through the elicitation of induced systemic resistance against pathogens of plants; hence, these bacteria can indirectly promote the plant growth [117,465,487,668]. *Bacillus thuringiensis (Bt)* is a unique soil bacterium that is gram-positive aerobic or facultative spore-forming and is included in the genus *Bacillus*. *Bt*-related studies are mostly focused on its insecticidal activity due to its entomopathogenic properties. *Bt* regarding its ability to interact with plants [128]. It was also reported that *Bt* can successfully colonize cabbage, cotton, soybean, and rice as an endophyte [31,475,480], Appendix 1.

The insecticidal toxins (Cry toxins) are usually expressed as δ -endotoxin and specifically act on some pest insect species [37,38,291]. Genes encoding these toxins are termed cry genes [127,533]. One significant common feature of the cry genes is that they are expressed during the stationary growth phase. These proteins start to appear during the 3rd phase of sporulation and persist until end of the 7th phase [91,268]. Some *Bt* strains produce non-parasporal insecticidal proteins during vegetative growth termed VIP (Vegetative Insecticidal Proteins). Generally, bacterial strains that have useful effects on plant growth are considered PGPB [289,389]. PGPB are beneficial microorganisms that help plant development [e.g., by producing indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate-deaminase (ACC-deaminase), phosphate - solubilizing enzyme (PSE), and siderophores (SD)], exhibit antimicrobial activity against plant pathogens (e.g., by producing bacteriocin, zwittermicin, fengycin, chitinase, and cell wall-degrading enzyme reported by scholars Sharma & Saharan [546], Jouzani et al., [289], Raddadi et al., [486].

Phytohormones have important functions in plant growth and development as regulators and signals. They are produced by bacteria that colonize plant roots and play key roles in plant growth, plant pathology, and plant-microorganism interactions [225,486,538]. IAA is a phytohormone of the auxin class and is the most physiologically, biochemically, and genetically studied plant growth hormone [146,486]. Some *Bt* strains colonize plant roots and have plant growth-promoting properties [32,44,227,289,389,546].

Praça et al., [475] emphasized that effective colonization of *Bt* on the surface of seedling roots can affect physiology of host plants and that this bacterium may act as a growth. Biological fertilizer can

be defined as a substance that increases a plant's mineral nutrient intake and transportation when applied to seed and contain viable microorganisms that can be found on the plant surface or in the soil, rhizosphere colonies, or plant interior [486,651].

Although phosphate (P) is present in high amounts in many types of soils, it is an important limiting factor of plant growth.

Phosphate solubilization can be improved through various mechanisms, such as hydrolysis or processes involving enzymes like phosphatases and phytases Matos et al., [365]. *Bacillus spp.* are known as one of the most significant phosphate-solubilizing bacteria (PSB) Behera et al., [61], Abdallah et al., [3] PSB convert the nonsoluble phosphate to the soluble form by enzymatic activity reported by Fitriatin et al., 2014 (Table 9) (Figure 6).

Table 9: Cassava Inoculants: Biofertilizer and Biostimulator phosphate solubilizing bacteria [701].

Phosphate solubilizing bacteria	Plant growth promoting traits	Cassava Inocula	References
<i>Pseudomonas sp.</i>	ACC deaminase, IAA, siderophore	OBD-Biofertilizer	Poonguzhali et al., [474]
<i>Bacillus subtilis</i>	IAA, siderophore, antifungal activity	OTAI AG®	Singh et al., [564]
<i>Pseudomonas fluorescens</i>	ACC deaminase	OBD-Biofertilizer	Shaharoon et al., [544]
<i>Acinetobacter sp.,</i>	ACC deaminase, IAA, antifungal activity,	OTAI AG®	Indiragandhi et al., [277]
<i>Pseudomonas sp.</i>	N ₂ - fixation	OBD-Biofertilizer	
<i>Enterobacter sp.</i>	ACC deaminase, IAA, siderophore solubilization	OTAI AG®	Kumar et al., [319]
<i>Pseudomonas jessenii</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization	OTAI AG®	Rajkumar et al., [493]
<i>Pseudomonas aeruginosa</i>	ACC deaminase, IAA, siderophore	OBD-Biofertilizer	Ganesan, et al., [213]
<i>Pseudomonas sp.</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization	OBD-Biofertilizer	Rajkumar et al., [493]
<i>Azotobacter sp., Mesorhizobium sp.,</i>	IAA, siderophore, antifungal activity, ammonia	OBD-Biofertilizer	Ahmad et al., [14]
<i>Pseudomonas sp.,</i>	production, HCN	OTAI X ®	
<i>Bacillus spp. chromium reduction, metal solubilization</i>	IAA, siderophores, ammonia production, HCN,	OBD-Biofertilizer	Wani et al., [671]; Wani et al., [672]; Ahmad et al., [14]
<i>Bacillus subtilis</i>	IAA	OTAI AG®	Zaidi et al., [701]
<i>Pseudomonas sp., Bacillus sp.</i>	IAA, siderophore	OTAI AG®, OTAI X®	Rajkumar et al., [492]
<i>Pseudomonas putida</i>	antifungal activity, siderophore, HCN	OBD-Biofertilizer	Pandey et al., [456]

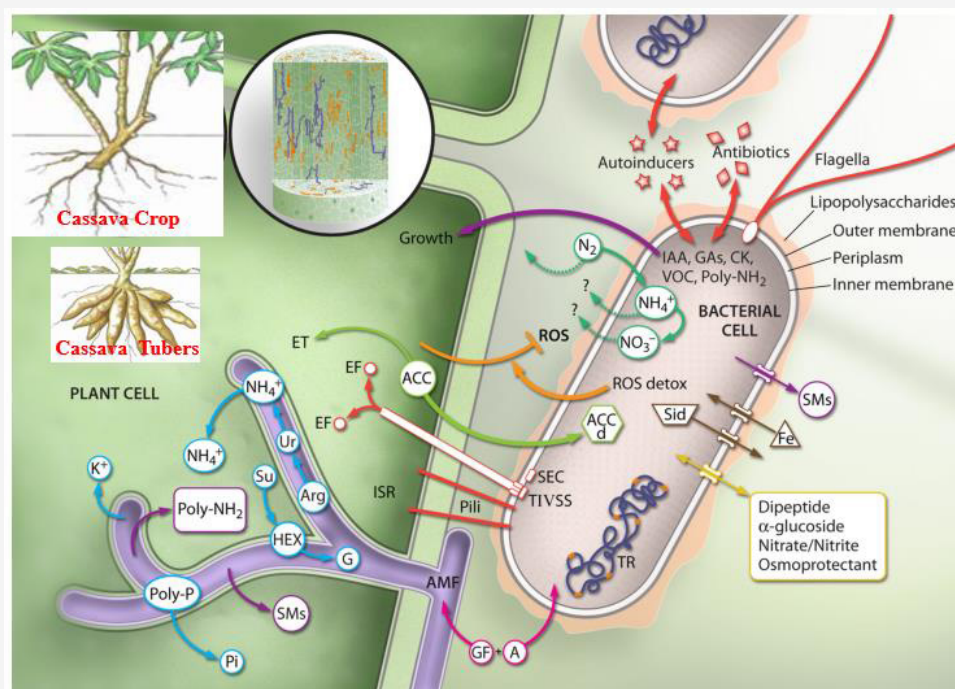


Figure 6: Beneficial properties of endophytes on the cassava crop adapted from Hardoim et al., 2015.

*Endophytes high potential as a less exploited resource in sustainable agriculture.

Cassava cultivar phytoremediation and Re-generative agriculture

Alves (2002) stated that cassava was a subsistence crop, grown by resource poor, small-holder farmers for land optimization techniques for crop failure in the tropics and low inputs crops. The future of cassava is to improve cultivar development increase crop yield, improves value chain development; income; soil health and yield and improve cassava cultivated on marginal soils [694]. Hillocks [18], suggested that the observed increase in acreage is related to declining soil fertility levels in Africa for chemical fertilizer application. According to FAO (2006), average cassava yields in Africa have gradually increased from 6 to 10 t/ha over the past five decades.

At present, the average African farmer harvests approximately 20% less cassava per hectare than the world average 12.2 t/ha due to no or low fertilizer inputs instead of addition of soil inputs that can yield 25-32 tons/ha [448] and there is the need to apply supplementary nutrients for sustainable crop production.

Howler (1990) earlier stated that large bulk of foliage are created by the action of nitrogen and consequently an extensive assimilating area, a pre-requisite for the good development of the roots. Roots development per plant is attributed to metabolites promotes the photosynthetic organs in the plant to produce and make available more assimilates to the root and increase the yield of cassava [711]. Biofertilizer application to crop cultivation and the role of PGPR is narrated in Figure 6 and adapted from Hardoim et al., 2015.

The cassava crop plants inoculated beneficial microorganisms significantly improve plant growth based on microorganisms in the biofertilizer inoculated to elaborate mechanisms of action in Tables 2 and 8. In Figure 6, bacteria (orange) and fungi (purple), can colonize the internal tissues of the plant (middle panel). Once inside the plant, the endophytic bacteria and fungi interact intimately with the plant cells and with surrounding microorganisms (large panel). Endophytic fungi, represented here as *arbuscular mycorrhizal* fungi (AMF) (lilac), might form specialized structures, called arbuscules, where plant-derived carbon sources, mainly sucrose (Su), are exchanged for fungus-provided phosphate (Pi), nitrogen (NH⁴⁺), and potassium (K⁺) elements (blue). Plant cytoplasmic sucrose is transported to the peri arbuscular space, where it is converted to hexose (HEX) to be assimilated by the fungus. Hexose is finally converted to glycogen (G) for long-distance transport reported by Hardoim et al., (2015).

The production of secondary metabolites is undoubtedly the major mode of action amongst endophytes. Endophytes are regarded as a micro-organism that lives in plant tissues partly or in all of their lifecycle, classified as beneficial, neutral and or detrimental depending on the kind of interaction with their host plant and example, *mycorrhizal fungi* and *rhizobia* are regarded as the beneficial microbes. Endophytes possess the ability to control

the pathogens of plant, insects and nematodes [311,509] xenobiotic degradation [442,444,445]. The emerging use of endophytes-based nanoparticles as value added materials [446,227] has showed promising results for future drug development. In the near future, the application of endophytes may revolutionize drug formulations like the pharmaceutical starch from cassava. Host plants can be induced to produce required metabolites of interest such as those used in drugs for treating cancer. Endophyte(s)-based bioformulations applied on seeds or aerial parts will be far more effective because once the microbe is inside the plant tissue, it will not face the competition of other soil microbes, which is common in the case of rhizosphere microbes (Table 9).

Endophyte-based bioformulations for remediation of contaminated soils, pollutants and biodegradation (Peng et al., 2013 and Muponda, 2014) that exhibit their symbiotic responsibilities to its host producing metabolites (Kumar et al., 2014 ; Gao et al., 2015) and support root development and access to nutrients (Tan et al., 2001) protect the plant from desiccation and from insects as well as parasitic fungi (Taghavi et al., 2011); root-knot entomopathogenic microbes (nematodes) treatment Elmi et al., 2000 ; Sikora et al., 2008 ; Hirose and Murakami (2011) illuminated in Figure 7.

Biofertilizer: Remediation-to-Biofuel sustainable development

Germain et al., 2006 studied the degradation of herbicides with bacterial endophytes (*Pseudomonas sp.*) and reported that there was no sign of accumulation of the herbicide in the plant tissue and no sign of phytotoxicity, unlike the uninoculated plant. The use of endophytes capable of degrading environmental contaminants in addition to the specific plants could offer an efficient, economic and sustainable remediation technology. Cassava crop new development cultivars could be used for phytoremediation of polluted soils (hydrocarbon) base on the report by Otaiku [446], that cassava cultivation in hydrocarbon producing communities of Niger -Delta have food security challenges because of impacted soils nutrients. Applied biofertilizer to polluted soils will open a new research future development for roots crops re-generative agriculture in Niger Delta called remediation-to-biofuel (cassava crop harvested converted to bioethanol) sustainable development. This paradigm shifts for polluted degraded soils called pollution construct, remediation, restoration and re-use (PC3R Technology) where microbial inocula in Tables 1 and 9 are applied for the xenobiotic biodegradation [442,444]. PC3R technology encapsulate genetics, bioremediation, phytoremediation, re-generative agriculture other techniques (Phyto stabilization, phytovolatilization, rhizofiltration, etc.). PC3R genetic studies using endophytes and omics techniques improved cassava cultivar gene carrier affirmed by scholars [535,539]. The genetically engineer cassava cultivar and microbial endophytes which will serve as protection for the host like cassava crop for remediation-to-biofuel development and convert remediated polluted soils in the tropics the bio-ethanol economy (waste-to-wealth project). The PC3R technology application

includes are xenobiotics (munitions waste) biodegradation [442-445], heavy metals, POPs and high molecular weight pesticides with their recalcitrant, bioaccumulation and bioconcentration properties, that generally defy conventional remediation practices and techniques [353,680]. The limitation of phytoremediation is often as a result of the toxicity of these chemicals or their toxic end products in plants [370,622] can be ameliorated through endophyte-assisted phytoremediation [267,576] and endophytic microbes activity [30,535] by incorporating into plant endophyte those natural microbe ability to conjugate with each other by means of movable DNA elements (vectors) between microbial populations [29] Case study report the introducing bacterial genes pTOM-Bu61 involved in the metabolism of toluene and TCE biodegradation [576].

Crude oil had variable effects on the microbial biomass [163] weakens soil microbe's activity influences plant root development [164], soil water absorption by plants [33], biotoxicity [33], soil structure, water stress and nutrients deficiencies [434] and decline in crop performance [200]. as elevated accumulation has direct consequences to man and ecosystem [8]. The low pH of the soil could explain the presence of cyanogenic glycosides in the cassava effluent contaminated soil. Low clay content was reported as soil conditions that increase cyanide mobility. The biodegradability of cassava effluent impacts on the physicochemical characteristics on soil dynamics and structure was reported by scholars the microbial contents isolated from the studies areas of Niger Delta, Nigeria was similar to the biofertilizer microbial inocula [36,167,279,303,436].

Biofertilizer biosafety

Populations of microorganisms applied to the environment commonly decline to a density naturally sustainable within that environment, often to undetectable levels. Plant-associated microorganisms introduced as biocontrol agents into the rhizosphere or phyllo sphere, the population of the microbial biocontrol agent declines to background levels when the supporting plant dies, and it must be applied again with the next planting of that crop [125,595], this promotes re-generative agriculture for modern agriculture. Evidence shows that the effects are short-term and subtle and that non-target populations stabilize relatively quickly after application is discontinued [93]. Studies have confirmed that plant-associated microorganisms introduced into soil remain virtually in the row where introduced and decline to undetectable populations soon after and sometimes before the supporting plant completes its life cycle [195] and see the narrative in Figure 6 and the challenge can be corrected deploying PC3R technology.

Impacts of Genomics on Cassava Development

Cassava is vegetative propagated through stem cuttings, and its growth cycle is longer than 10 months. Cassava breeding is hampered due to the high degree of genetic heterozygosity, genetic overloading, serious separation of progeny, few flowers, low pollen fertility, self-incompatibility, and low fruit set rate [101]. Genetic

engineering shows great potential in cassava genetic improvement and can compensate for the limitations of conventional breeding for cassava. Programs, such as HarvestPlus and BioCassava Plus, have made remarkable achievements by transforming traditional breeding into molecular breeding [529]. Schopke et al., (1996) reported milestone for cassava molecular breeding.

The commonly used methods for the genetic transformation of cassava include *Agrobacterium*-mediated gene delivery and particle bombardment. *Agrobacterium* is one of the microbes in the biofertilizer reported by Otaiku et al., [448] (Paper 1). The explants used for transformation include somatic cotyledons and FEC. Usually cassava transformation is carried out using FEC and/or embryogenic suspensions by *Agrobacterium tumefaciens* or particle bombardment. Gonzalez et al., [229] successfully transformed FEC of the West African cultivar 60444 (also known as TMS60444) with the *Agrobacterium tumefaciens* strain ABI. The most prominent advantage of the *Agrobacterium*-mediated transformation system is the availability of a large number of transgenic plants; thus, it is the most widely used method for cassava genetic engineering. Climate change vulnerabilities necessitated the breeding of new cassava varieties with increased nutrition, high stress resistance and starch content [101] using the genetic engineering in germplasm innovation by improving specific traits without changes in other important traits using genome editing technology'-omics' tools have led to intensive cassava starchy storage root development, starch accumulation, health-promoting components (e.g. beta-carotene), and stress response and regulation [529,693].

Genetic development using *Agrobacterium*-mediated transformation protocols for TMS60444 friable embryogenic callus (FEC) by Liu et al., [338]. In Table 7 and Figure 7 several genetic transformation systems of farmer-preferred cassava cultivars have been successfully established using African, Asian and South American cultivars they still need to be optimized due to cultivar dependence. The first successful *Agrobacterium*-mediated cassava genetic transformation that was reported was from the Potrykus laboratory at ETH Zurich in 1996 using *Agrobacterium* strains harboring different binary vectors (e.g. LBA4404 (pTOK233) and others to transform cassava somatic cotyledons. Sarria et al. (2000) successfully transformed an herbicide (phosphinotricin, ppt)-resistance gene into the cotyledons of cassava MPer183 by an *Agrobacterium*-mediated method and obtained stable transgenic plants resistant to Basta spray (at concentrations of 200 mg/L).

Also, Siritunga & Sayre (2003) developed transgenic cassava with a lower cyanide content using MCol2215 cotyledon explants). Analysis of the transgenic plants revealed the integration of the target gene into the genome and its expression at the transcript level. Field experiments showed that the transgenic plants had significantly delayed leaf senescence, drought tolerance, and altered cytokinin content in their leaves. Transformation efficiency was enhanced with low *Agrobacterium* density during co-cultivation and co-centrifugation of FEC with *Agrobacterium*, possibly facilitating

plant regeneration [105,428]. Application of TALEN and CRISPR/Cas9 systems to mutant target genes is now in demand in cassava [683] and the utilization of this tool in cassava is still in its infancy and no published reports involving genome editing of cassava to

date, although several laboratories are working on different genes and traits. Based on draft cassava genome sequences [476,670]. Cassava breeders are at the turning point of trait improvements in important root crops.

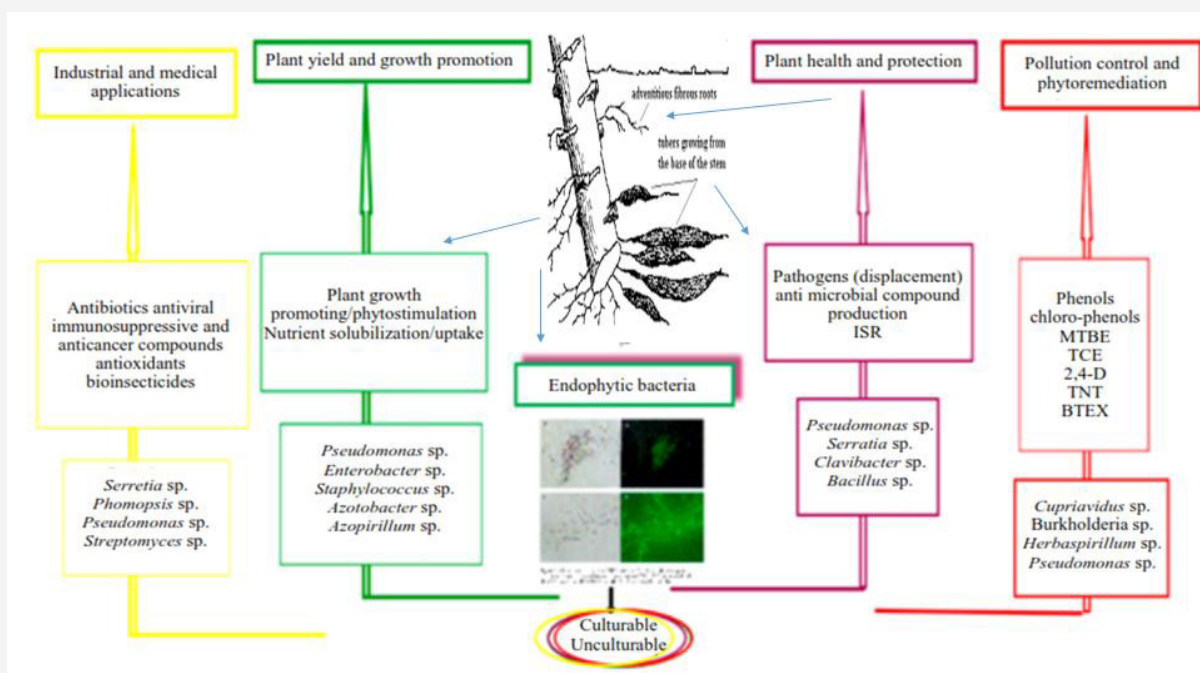


Figure 7: Schematic diagram of the different plant–bacterial endophyte interactions in the biofertilizer applied [448] that for cassava soil microbiome soil microbiome engineering, genetic and sustainable agroecosystems adapted from Ryan et al., [509].



Figure 8: Biology of tropical root crop cassava (*Manihot esculenta* Crantz) Liu et al., [338]

- (A) Six-month-old cassava plants in field. (B) storage roots harvested from 10-month-old cassava plants.
- (C) inflorescence of cassava showing opened female flowers and male flower buds. (D) cassava fruits.
- (E) cassava seeds. (F) plantlets germinated from seeds. (G) a stem cutting used for planting.

The candidate gene identification study is still dependent on traditional methods, such as Cdna library screening under stress [521] or transcriptome analysis under different treatments [619] or development stages [693]. Researchers are seeking specific

ways to study massive microarray data [619,693], RNA-seq [340,350] and even genome sequencing [670]. *Agrobacterium* sp is microbe used in the biofertilizer formation as inocula reported by Otaiku et al., [448]. Cassava mosaic disease (CMD), one of the

major viral diseases in Africa, is responsible for yield reductions of 20–95% in certain areas [322] and cassava brown streak disease (CBSD) result in great loss of cassava production in sub-Saharan Africa and the Indian subcontinent [323]. In the model cassava cultivar TMS60444, CMD-resistant transgenic cassava has been developed using both antisense and dsRNA technology [633-

635,688,707]. To develop CMD-resistant cassava, African breeders used resistant germplasm of wild cassava relatives (*Manihot glaziovii*) to obtain new cultivars of cassava resistant to CMD, which have been widely adopted in the major epidemic regions, reduced, and cassava cultivation has gradually been recovered (Figures 8 & 9).

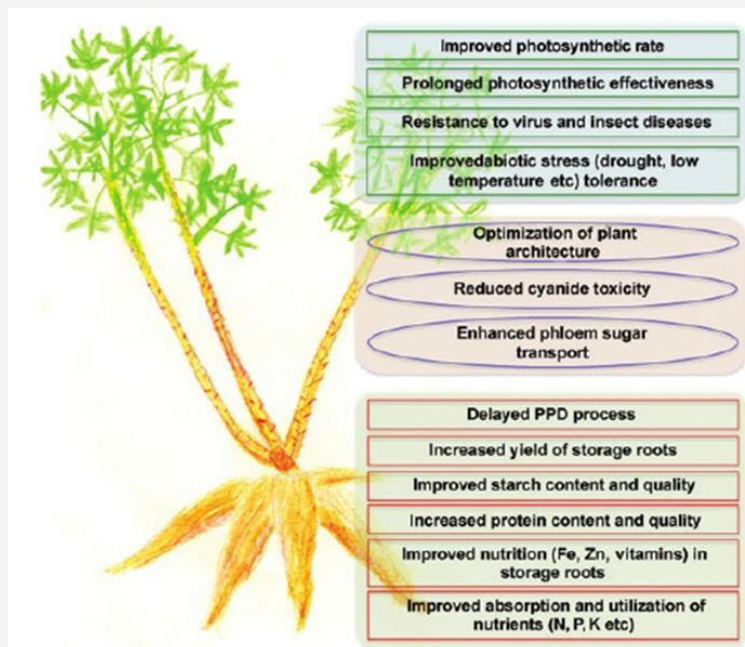


Figure 9: Important agronomic traits for cassava genetic engineering. PPD, post-harvest physiological deterioration adapted from Liu et al., [338].

However, this variety comes from West Africa and is not resistant to African cassava mosaic disease (CMD) or cassava brown streak virus disease (CBSD). It is necessary to develop FEC-based transformation systems in other cultivars that are preferred by farmers and the industry. In this regard, several laboratories have made unremitting efforts by investigating various genotypes for FEC production, and success has been achieved in several cultivars, such as TME7, Ebwanatereka, TME1, TMS91/02327, Rosinha, and Buja Preta [268]. Transgenic technology, as a powerful tool, also plays an important role in obtaining virus-resistant cultivars [633]. Chellappan et al., (2004) used pILTAB9001 and pILTAB9002 harboring the wild-type and mutant AC1 genes of ACMV-Kenya, which were regulated by the cassava vein mosaic virus promoter and the pea Rubisco terminator for the production of transgenic TMS60444 lines with increased resistance to mosaic disease. Insecticide proteins, such as *Bt* Cry proteins, protease inhibitors, α -amylase inhibitor, and plant lectins, could pave the way for insecticides, as a high expression of these products in transgenic cassava might be useful for increased insect resistance.

Transcriptomic studies revealed a rapid change in cassava genes after infection by this disease [343]. Using the leaf senescence-induced promoter, SAG12, to express the *ipt* gene, transgenic cassava showed not only prolonged leaf life, but also improved

resistance to drought stress [705]. Transgenic plants also showed altered composition of amino acids and reduced cyanide content. Therefore, a transgenic approach to cassava protein enhancement is practical and is a useful way to reduce protein deficiency in poverty-stricken regions [638]. Biofortification of cassava by BioCassava Plus and of Harvest Plus advanced breeding and the nutrition issues of zinc, iron, and vitamins A and β -carotene-rich cassava [269,529]. Cassava accumulates a large amount of starch in its storage roots and its native starch has many shortfalls such as low solubility and retrogradation. To overcome these limitations, native starch is often modified chemically, physically or biotechnologically [292]. The challenge in most countries are not yet equipped to approve transgenic waxy cassava through the regulatory process. Storage roots are the cassava plant's main commercial product, and therefore understanding storage root development is key to improving starch accumulation and root production. Jørgensen et al. (2005) conducted a similar experiment by RNAi and found that the cyanogenic glucoside contents of cassava storage roots were reduced by 92%. White et al. [599] reported that at transcript level, the hydroxy nitrile lyase content in roots is only 6% of that in leaves. The overexpression of hydroxy nitrile lyase can reduce the acetone cyanohydrin content of roots, thus accelerating the detoxification process. To achieve this goal, the cDNA of the gene encoding hydroxy nitrile lyase was cloned between the CaMV 35S

promoter and the pea ribulose biphosphate carboxylase terminal sequence, and transformed into MCol2215, Appendix 1.

Comparative proteomes also show that root formation may be influenced by metabolic and regulatory processes in cassava leaves [391]. Through AFLP-based transcript profiling, Sojikul et al. [569] found that four genes in the MeKD family exhibit critical expression in initiation and early stage development of the cassava storage root. An estimated 26% of total cassava production is lost owing to post-harvest physiological deterioration (PPD) reported by Sayre et al., [529]. Genetic improvement can potentially delay or inhibit PPD in cassava and PPD process differs among cassava genotypes and PPD-susceptible HMC-1 variety and PPD-tolerant experimental hybrid AM206-5 were significantly different in PPD level and in secondary metabolic synthesis [523] and engineering of cassava storage roots with prolonged shelf life. Among the significantly impacted pathways, glycolysis / gluconeogenesis was the most vital one [693], suggesting the importance of carbon mobilization during storage root development. Recent studies based on cDNA microarray-based transcriptomes [502] and iTRAQ proteomics [453] showed that the onset of PPD is related to signal transduction pathways involving multiple enzymes, cellular processes including

reactive oxygen turnover, signal transduction, stress response, metabolism and biosynthesis.

Reactive oxygen species (ROS) production and turnover in the root is closely associated with PPD. O_2^- and H_2O_2 quickly accumulate in the root within 15 minutes after harvest, with peak levels at 4hrs and 24hrs, respectively [502]. Accumulation of ROS in the storage root can be observed directly by fluorescent probes (singlet oxygen sensor green, dihydroergotamine 123), which indicates oxidative bursts during the PPD process [686]. Zidenga et al., [712] reported that PPD is cyanide-dependent, probably because of a cyanide-dependent inhibition of respiration. Post-harvest physiological deterioration has a close relationship with reactive oxygen species (ROS). Using the cDNA-AFLP technique, Beaching's laboratory has analysed the proteins and enzymes affecting PPD, most of which are involved in signal transduction, ROS, cell wall repair, programmed cell death, metabolite transport, signal transduction, and a series of biological processes [502]. The upregulation or downregulation of key enzymes or factors in the PPD pathway by the overexpression or RNAi might effectively slow or reduce the occurrence of PPD (Figures 10 & 11).

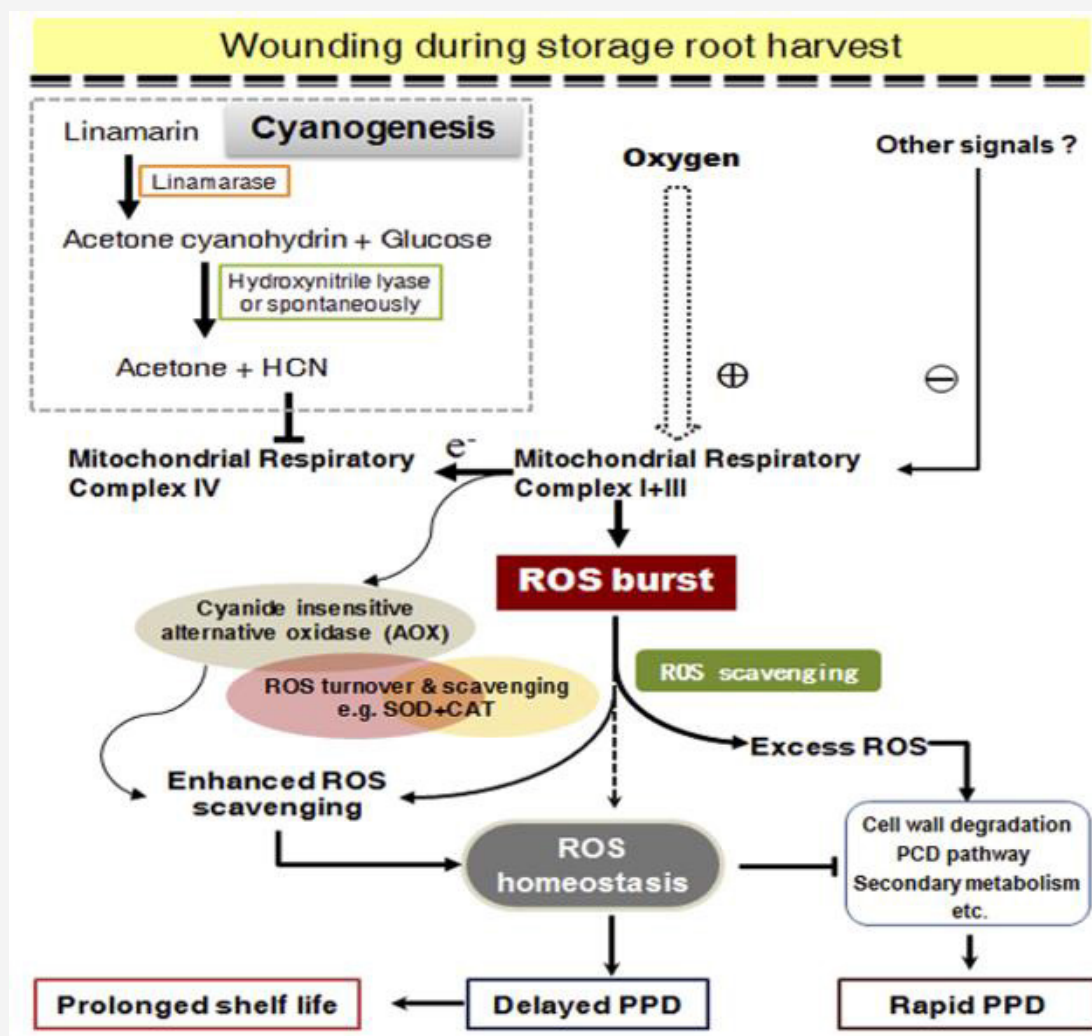


Figure 10: Regulatory mechanism of post-harvest physiological deterioration in cassava storage roots and adapted from Zhang et al., [183].

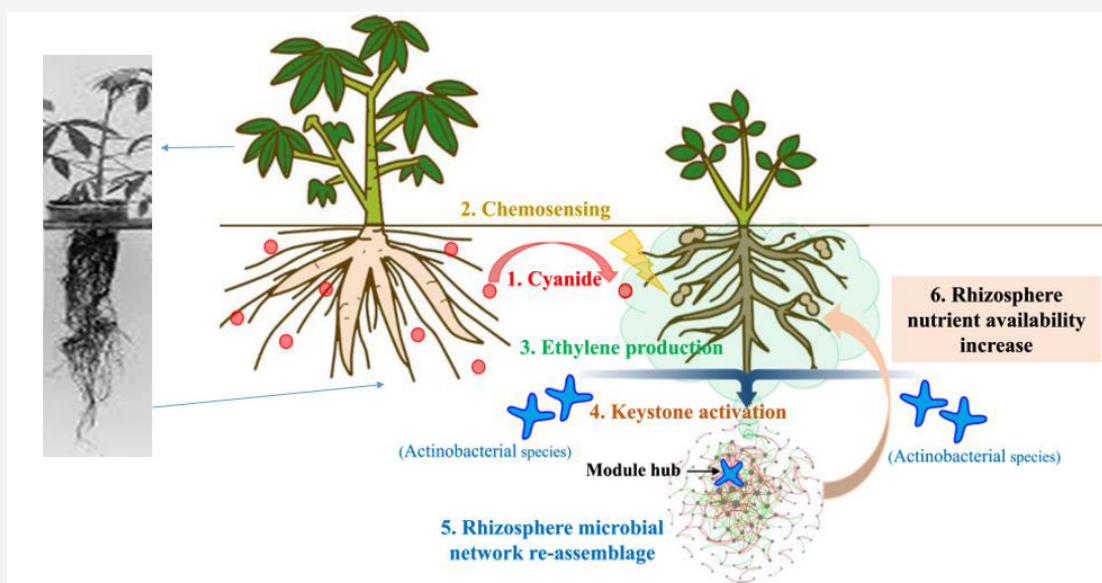


Figure 11: The overview of the mechanism by which peanut mediates rhizosphere microbiota to increase available nutrients in the cassava-peanut intercropping system. Cyanide exposure belowground induces ethylene production in peanut roots, and ethylene mediates actinobacterial species to reshape rhizosphere microbiota, which promote peanut seed production by increasing available nutrient content, adapted from Chen et al., [115].

The production of reactive oxygen species (ROS) is an inevitable consequence of an aerobic lifestyle. ROS participate in signaling pathways in plants, animals, and fungi [189,536,606] and even in interspecies communication [606]; and it has also been proposed that they play a role in the development of multicellularity [328]. ROS is in fact beneficial to longevity through the adaptive mechanism called hormesis [455,506]. During hormesis, low doses of stress or toxin induce mechanisms that protect the organism against this stressor and evoke cross adaptation to other stresses. Lethal attacks from bacterial and viral species also result in ROS production in target cells. As a model prey or target organism, *Escherichia coli* can be killed by the T6SS activities of a number of bacteria including *Pseudomonas aeruginosa* and *Acinetobacter baylyi* ADP1.

Cassava Sustainable and Ecological Intensification

The growing concern for ecologically grown foods due food safety and security, environmental protection, biodiversity and human health [144,178,530]. The distribution of cassava is related to climatic conditions and most of the cassava producing regions are between Tropic of Cancer and Tropic of Capricorn and elevations up to 2300m above mean sea level [18]. Cassava, intermediate photosynthetic pathway between the C3 and C4 [172] can be adaptive "efficient" C4 pathway of higher plants [169]. Cassava can yield 80t/ha of fresh tuber (experimental farms) and 40t/ha of fresh tuber improved cultivars (commercial fields); productivity in seasonally dry and semi-arid environments without fertilization is much less [173,481]. Average yield of fresh tuber of cassava varies from 1.13 to 32.68 t/ha among 104 cassava growing countries with a global average yield of 11.80 t/ha, which is far below the potential yield of 80–100t/ha [94]. A large yield

gap exists between yield potential and farmers yields due to lack of adoption of improved varieties, better agronomy and soil fertility management [170,171,186]. Because of its inherent tolerance to prolonged drought and infertile soils [172], cassava production is expanding into more marginal lands and drier environments for subsistence [186,515].

Secondary nutrients influence cassava cultivation, namely, calcium, magnesium and sulphur reported by [306,478,394,395]. Liming increased the tuber yield and starch content and decreased the HCN content [405]. Mohankumar & Nair, [395], Howeler [264] reported that Magnesium deficiency was observed in cassava cultivated in Oxisols, Ultisols, Inceptisols and Entisols (Application of Sulphur at 50kg/ha resulted in a significantly higher tuber yield and starch content and a lower HCN content, total protein and methionine contents. Cerilles [100] reported significance of organic cassava mitigating the effects of climate change in the Philippines. Zhongyong et al., [711] reported that cassava bio-organic fertilizer treatment promoted the leaf and stem growth, increased the chlorophyll content, photosynthesis of leaves, improved the physiological metabolism of cassava, transfer of photosynthates to storage roots and increased yield and starch content in the storage roots of cassava. Organic nutrient management also resulted in greater N and K uptake over chemical system, Also, similar impacts was reported for compost and inoculated with mixed biofertilizers [552] which affirmed the report by Otaiku et al., [448]. Radhakrishnan [494,495] 2019 reported ultimately reflected in tuber yield under organic management (27.26 t/ha). Organic and inorganic nutrient sources had significant effect on quality of cassava tubers [451]. Cyanogenic glucoside levels decreased with the application of organic fertilizers while inorganic fertilizer increased the level of

cyanogenic glucoside and decreased the phytochemical contents in both the leaves and tubers. Nutritional quality was improved by organic fertilization with significant effects on antioxidant activity and phenolic metabolites in cassava reported by [451]. Omar et al. [452] also observed that application of organic fertilizers like vermicompost was favorable for enhancement of antioxidants and total phenolic acids in cassava leaves that contributed to nutritional value of leaves used as vegetable.

Conclusion

The ability of endophytes to colonize every plant tissue has led to the opportunity of using the microorganism in agriculture and environmental biotechnology for sustainable development like the biofertilizer technology. The different constraints in biofertilizer technology can be mitigated [611,655] using nano-biotechnology and value-added materials [446], Table 13 as an alternative to chemical fertilizer. Cassava adaptation exhibited by plants growing in an extreme environment and expressing genes

for adaptation can be improved using genetic engineering to become phytoremediation xenobiotic cultivar for pollution control and remediation-to-biofuel regenerative agriculture because of the rhizosphere microbe's potentials like *Bacillus Pseudomonas*, and *Agrobacterium* species with their outstanding root-colonizing ability, and Horizontal Gene Transfer (HGT), catabolic flexibility and ability to produce a wide range of metabolites for ecological services and agrosystems management to improve the cassava crop sustainable development goals.

Acknowledgement

None.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest [Appendix 1 (Figure 12)] [Appendix 2 (Tables 10-13)].

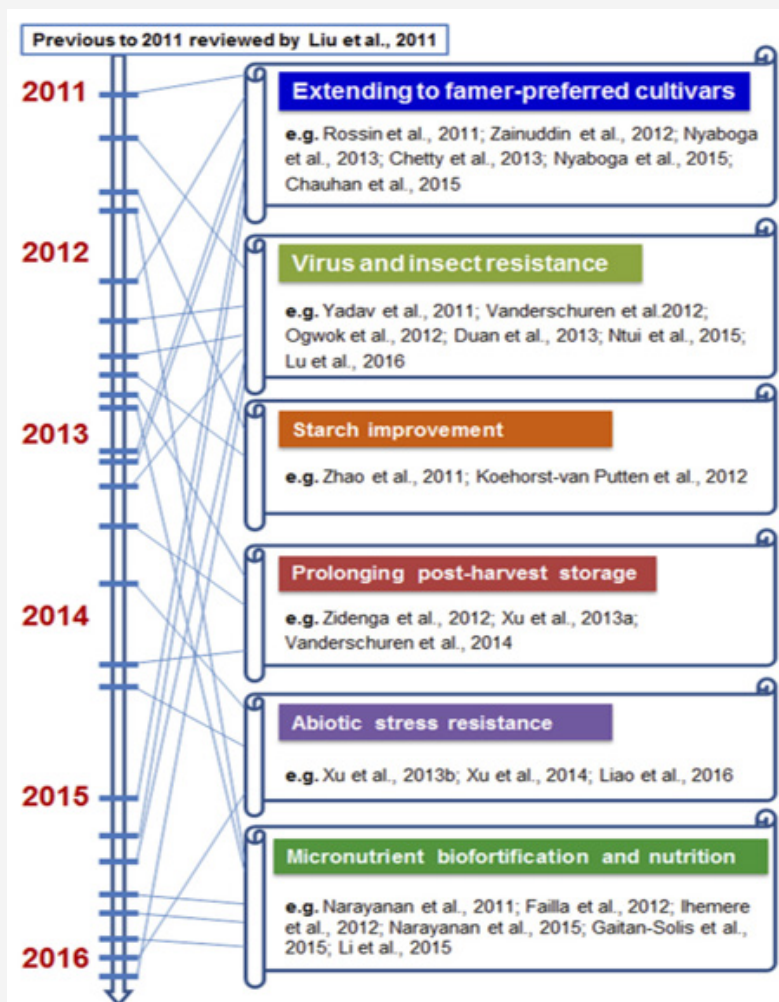


Figure 12: The development of cassava genetic engineering for technology adaption to farmer preferred cassava cultivars and value-added traits in the last 5 years, adapted from Zhang et al., [709].

Table 10: Advantage and disadvantage of using biofertilizer.

S. No.	Advantage	Disadvantage
1	The nutrient supply is more balanced, which helps keep plants healthy	They are comparatively low in nutrient content, so a larger volume is needed to provide enough nutrients for crop growth
2	They enhance soil biological activity, which improves nutrient mobilization from organic and chemical sources and decomposition of toxic substances	The nutrient release rate is too slow to meet crop requirements in a short time; hence, some nutrient deficiency may occur
3	They enhance the colonization of mycorrhizae, which improves phosphorus supply	The major plant nutrients may not exist in organic fertilizer in sufficient quantity to sustain maximum crop growth
4	They enhance soil structure, leading to better root growth	The nutrient composition of compost is highly variable
5	They increase the organic matter content of the soil, thereby improving the exchange capacity of nutrients, increasing soil water retention, promoting soil aggregates and buffering the soil against acidity, alkalinity, salinity, pesticides and toxic heavy metals	The cost is high compared to chemical fertilizers.
6	a. They release nutrients slowly and contribute to the residual pool of organic nitrogen and phosphorus in the soil, reducing nitrogen leaching loss and phosphorus fixation; they can also supply micronutrients	Short shelf life, lack of suitable carrier materials, susceptibility to high temperature and problems in transportation and storage are all biofertilizer bottlenecks that still need to be solved in order to promote
	b. They encourage the growth of beneficial microorganisms and earthworms	They encourage the growth of beneficial effective inoculation
	c. They help to suppress certain soil-borne plant diseases and parasites	

Table 11: Horizontal Gene Transfer in Soil and the Rhizosphere: Impact on Ecological.

N/B	Factors Effects	Effects/explanation
1	Drying and sieving	Disturbing the soil results in poor survivability of strains and ultimately poor conjugation
2	Earthworm activity	Varying influence, depending upon the depth at which earthworms are present
3	Nutrient availability	Nutrient availability enhances survival and therefore gene transfer
4	Plant rhizosphere	High concentrations of root exudates and nutrients in the rhizosphere positively affect gene transfer
5	Selective pressure such as heavy metals or recalcitrant organics	In most of cases, heavy metals/recalcitrant organics enhance the number of transconjugants
6	Soil type	Soil variables such as types and amounts of clay, organic matter, pH, moisture, and incubation temperature influence gene transfer
7	Spatial separation between donor and recipient strains	Moisture content helps in movement and survivability of strains thus directly affecting gene transfer
8	Sterilization	Sterilization of soil increases transfer frequencies
9	Temperature	Higher transfer frequency observed at environmentally relevant soil temperatures

Table 12: Advantage and disadvantage of chemical fertilizer.

S. No.	Advantage	Disadvantage
1	Nutrients are soluble and immediately available to plants, creating a direct and fast effect	Overuse can result in negative effects such as leaching, pollution of water resources, destruction of microorganisms and beneficial insects, crop susceptibility to disease attack, acidification or alkalization of the soil or reduction in soil fertility, all of which cause irreparable damage to the overall ecosystem
2	The price is lower and more competitive than organic fertilizer, which makes it more popular with farmers	Oversupply of nitrogen leads to softening of plant tissue resulting in increased susceptibility to diseases and pests
3	They are quite high in nutrient content; only relatively small amounts are required for crop grow	a. They reduce the colonization of plant roots with mycorrhizae and inhibit symbiotic nitrogen fixation by rhizobia due to high nitrogen fertilization
		b. They enhance the decomposition of soil, which leads to degradation of soil structure
		c. Nutrients are easily lost from soils through fixation, leaching or gas emission and can lead to reduced fertilizer efficiency

Table 13: The different constraints in biofertilizer technology.

S. No.	Biofertilizer technology constraints	Example
1	Technology	1) Use of less efficient microbial strains and carrier materials
		(2) Low quality and short shelf-life of microbial inoculants
		(3) Lack of technically qualified personnel
2	Infrastructure	1) Non-availability of suitable production facilities like equipment, space, storage, etc.
3	Financial and Marketing	1) Non-availability of sufficient funds
		(2) Less return by sale of products
		(3) Non-availability of right inoculant
		(4) Lack of retail outlets or market network for producers
4	Environmental	1) Seasonal biofertilizers demand
		(2) Soil characteristics
		(3) Simultaneous short-span cropping operations
5	Human Resources	1) Lack of appropriate training on production practices
		(2) Unfamiliarity on the quality of the manufactured product
		(3) Problem in adoption and unawareness of the benefits of technology by farmers
		(4) Ignorance on the environmental indemnities caused by continuous application of chemical fertilizer

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