

Review

Possible Roles of Rhizospheric and Endophytic Microbes to Provide a Safe and Affordable Means of Crop Biofortification

Yee-Shan Ku [†], Hafiz Mamoon Rehman [†] and Hon-Ming Lam ^{*ID}

Centre for Soybean Research of the State Key Laboratory of Agrobiotechnology and School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China; ysku@ymail.com (Y.-S.K.); hafizmamoonrehman@cuhk.edu.hk (H.M.R.)

* Correspondence: honming@cuhk.edu.hk; Tel.: +852-3943-6336

[†] The authors contributed equally to this article.

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Abstract: Biofortification has been used to improve micronutrient contents in crops for human consumption. In under-developed regions, it is important to fortify crops so that people can obtain essential micronutrients despite the limited variety in their diets. In wealthy societies, fortified crops are regarded as a “greener” choice for health supplements. Biofortification is also used in crops to boost the contents of other non-essential secondary metabolites which are considered beneficial to human health. Breeding of elite germplasm and metabolic engineering are common approaches to fortifying crops. However, the time required for breeding and the acceptance of genetically modified crops by the public have presented significant hurdles. As an alternative approach, microbe-mediated biofortification has not received the attention it deserves, despite having great potential. It has been reported that the inoculation of soil or crops with rhizospheric or endophytic microbes, respectively, can enhance the micronutrient contents in various plant tissues including roots, leaves and fruits. In this review, we highlight the applications of microbes as a sustainable and cost-effective alternative for biofortification by improving the mineral, vitamin, and beneficial secondary metabolite contents in crops through naturally occurring processes. In addition, the complex plant–microbe interactions involved in biofortification are also addressed.

Keywords: biofortification; plant growth-promoting bacteria; endophytes; arbuscular mycorrhizal fungi; sustainable agriculture; plant–microbe interaction

1. Introduction

It is critical for modern agriculture to continue feeding the expanding world population. To support the ever-growing populations, strategies have been employed to maximize the biomass production. One of the famous examples is the “green revolution” which has significantly boosted crop yields to combat hunger. Besides the yield in biomass, the nutritional values of crops are another important consideration for providing proper nutrition. Apart from caloric intake and macronutrients such as nitrogen (N), phosphate (P), and potassium (K), humans also depend on food crops for obtaining certain micronutrients. The malnutrition due to insufficient dietary intake of micronutrients such as minerals and vitamins is regarded as the “hidden hunger”. It is predicted that the problem of hidden hunger will be intensified by climate change [1]. For example, it is predicted that the increasing anthropogenic carbon dioxide emission could intensify iron (Fe) deficiency because several crops were found to have lower Fe levels when grown under increased atmospheric CO₂ concentrations [2]. Fe deficiency is one of the most common forms of micronutrient deficiency worldwide [3]. FAO data

reveal that both hunger and “hidden hunger” in sub-Saharan Africa are of concern [4]. In other areas of the world, although the dietary energy intake is satisfactory, “hidden hunger” is still an issue to be addressed [4].

The enrichment of micronutrients in crops is known as biofortification, which has been a common practice for combatting “hidden hunger” [5–7]. Current strategies of biofortification include the application of organic and chemical fertilizers, breeding of elite germplasms, and metabolic engineering [8–11]. The advantages and the drawbacks of these strategies have been extensively summarized in previous reviews [8–11]. For example, breeding and CRISPR/Cas9 technologies can be used to enhance the nutritional values of crops but they are time-consuming, expensive, and are faced with numerous regulatory issues regarding genetically modified organisms (GMOs). Using soil microbes to increase the bioavailability of micro- and macronutrients in the soil is the cheapest, quickest, and most sustainable way to increase the concentrations of micronutrients in our diets while restoring soil health at the same time. In view of the environmental threats from the excessive use of organic and chemical fertilizers, the complexity of selecting suitable germplasms for breeding, and the hurdles to launching transgenic crops due to safety concerns and public acceptance [8–12], the use of microbes for biofortification has been suggested as a “greener” alternative for sustainable agriculture in recent years because it makes use of naturally occurring biological processes. Besides being “greener”, the use of microbes for biofortification provides unique benefits in some cases, such as the enrichment of vitamin B₁₂, which is only synthesized by bacteria and archaea but not plants. In this review, we will discuss several examples of microbe-mediated crop fortification to highlight the feasibility and benefits of this alternative approach to biofortification. Satisfying the dietary requirements of minerals and vitamins by human have been a concern. In a joint expert consultation between FAO and WHO, food-based approaches to meeting the requirements of Fe, Zn, vitamin A, folate, and vitamin C were discussed [13]. In this review, microbe-mediated biofortification of these micronutrients and other health-beneficial nutrients, including flavonoids and saponins, will be discussed. The use of both rhizospheric and endophytic microbes will be featured in examples of microbe-mediated biofortification in food crops. Examples of currently biofortified crops are summarized in Table 1, followed by the type of microbes used for the biofortification and their impacts on the crop plants. Figure 1 illustrates the complex processes of microbe-mediated biofortification. In addition, the suppression of pathogens by beneficial soil microbes and the influence on soil microbe communities by plants will also be discussed.

Table 1. Current examples of microbe-mediated biofortified crops.

Crop	Microbe	Type of microbe	Tissue	Impact	Reference
Blackberry	<i>Pseudomonas fluorescens</i> N21.4	Rhizospheric bacterium	Fruit	Improved flavonoid level	[14,15]
Brassica juncea	<i>Bacillus toyonensis</i> (MG430287), <i>Rhodococcus hoagii</i> (MG432495), <i>Lysinibacillus mangiferihumi</i> (MG432492), and <i>Lysinibacillus fusiformis</i> (MG430290)	Rhizospheric bacteria	Root, stem, and leaf	Increased Fe contents	[16]
Chickpea and pigeonpea	<i>Pseudomonas plecoglossicida</i> SRI-156, <i>Brevibacterium antiquum</i> SRI-158, <i>Bacillus altitudinis</i> SRI-178, <i>Enterobacter ludwigii</i> SRI-211, <i>E. ludwigii</i> SRI-229, <i>Acinetobacter tandoii</i> SRI-305, and <i>Pseudomonas monteilii</i> SRI-360	Rhizospheric bacteria	Grain	Improved contents of Fe (up to 18% and 12%), Zn (up to 23% and 5%), Cu (up to 19% and 8%), Mn (up to 2% and 39%) and Ca (up to 22% and 11%)	[17]
Chickpea	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	Arbuscular mycorrhizal fungi	Grain	Increased protein, Fe, and Zn contents	[18]
	Isolates of actinobacteria	Rhizospheric bacteria	Seeds	Increased Fe (10%–38%), Zn (13%–30%), Ca (14%–26%), Cu (11%–54%) and Mn (18%–35%) and Mg (14%–21%)	[19]
Lettuce	<i>Glomus fasciculatum</i>	Arbuscular mycorrhizal fungus	Leaf	Improved ascorbate level and fresh weight	[20]
	<i>Glomus intraradices</i> (Schenck and Smith) and <i>Glomus mosseae</i> [(Nicol. and Gerd.) Gerd. and Trappe]	Arbuscular mycorrhizal fungi	Leaf	Improved ascorbate level and fresh weight	[20]
Oil palm	<i>Hendersonia toruloidea</i>	Endophytic fungus	Leaf	Improvement of thiamine biosynthesis	[21,22]
Okra	<i>Azotobacter</i> sp. and cyanobacterium (e.g., <i>Calothrix</i> sp.)	Rhizospheric bacteria	Fruit	Increased concentration of Zn 60%–70%	[23]
Onion	<i>Rhizophagus irregularis</i>	Arbuscular mycorrhizal fungus	Bulb	Improved biomass production, the abundance of vitamin B1 and its analogues, and organic acids concentration	[24]
Pak choi	<i>Funneliformis mosseae</i> , <i>Glomus versiforme</i> , and <i>Rhizophagus intraradices</i>	Arbuscular mycorrhizal fungi	Shoot	Reduced concentrations of Pb (by 20.6%–67.5%) and Cd (by 14.3%–54.1%) in shoots	[25]

Table 1. Cont.

Crop	Microbe	Type of microbe	Tissue	Impact	Reference
Potato	<i>Glomus irregulare</i>	Arbuscular mycorrhizal fungus	Roots and tubers	Controlled the growth of <i>Fusarium sambucinum</i> and inhibited the production of the mycotoxin trichothecene 4, 15-diacetoxyscirpenol	[26]
Rice	<i>Bacillus sp.</i> SH-10 and <i>B. cereus</i> SH-17	Rhizospheric bacteria	Grain	Increased Zn concentration in grain (to ~31 from ~18 mg kg ⁻¹ in control)	[27]
	Cyanobacteria-based inoculants	Rhizospheric bacteria	Grain	13%–46% enhancement in iron and 15%–41% enhancement in zinc	[28]
	<i>Enterobacter sp.</i> ScCS20	Rhizospheric bacterium	Grain	Improved Zn level	[29]
	<i>Sphingomonas sp.</i> SaMR12	Rhizospheric bacterium	Grain	Improved Zn level	[29]
Rice-wheat cropping system	<i>Anabaena oscillarioides</i> , <i>Brevundimonas diminuta</i> , and <i>Ochrobactrum anthropi</i>	Rhizospheric bacteria	Grain	Micronutrient enrichment: Fe, Zn in rice, and Cu, Mn in wheat. Increased N-P-K contents and improved rice yield by 21.2% (compared to using the recommended dosage of chemical fertilizers)	[30]
Safed musli	<i>Glomus fasciculatum</i> , <i>Glomus intraradices</i> , or <i>Glomus mosseae</i>	Arbuscular mycorrhizal fungi	Tuber	Improved saponin level and improved biomass of roots and shoots	[31]
	<i>Piriformospora indica</i> and <i>Pseudomonas fluorescens</i>	Arbuscular mycorrhizal fungus and rhizospheric bacterium respectively	Micropropagated plants	<i>P. indica</i> enhanced the level of saponin in the plants. The co-inoculation of <i>P. indica</i> and <i>P. fluorescens</i> further enhanced the saponin level and also the contents of micronutrients including Fe, Zn, and Mn.	[32]
Snap bean	<i>Rhizobium etli</i> and <i>Glomus intraradices</i>	Rhizospheric bacterium and arbuscular mycorrhizal fungus	Seed	Increased yield, number of snap beans, and percentage of protein after N fertilization	[33]
Spinach	<i>Glomus fasciculatum</i> , <i>Glomus mosseae</i> , N-fixers (<i>Azotobacter chroococcum</i>), K solubilizer (<i>Bacillus mucilaginosus</i>), and P solubilizer (<i>Bacillus megaterium</i>)	Arbuscular mycorrhizal fungi and rhizospheric bacteria	Leaf	Augmented the concentrations of total phenolic compounds, flavonoids, and phenolic acids	[34]

Table 1. Cont.

Crop	Microbe	Type of microbe	Tissue	Impact	Reference
Strawberry	<i>Rhizophagus intraradices</i> , <i>Glomus aggregatum</i> , <i>Glomus viscosum</i> , <i>Claroideoglomus etunicatum</i> , and <i>Claroideoglomus claroideum</i> , and <i>Pseudomonas fluorescens</i> strain Pf4 (Pf4) and <i>Pseudomonas</i> sp. 5Vm1K (5Vm1K)	Arbuscular mycorrhizal fungi and Rhizospheric bacteria	Fruit	Co-inoculation increased fruit production, fruit size, and concentrations of sugars and ascorbic and folic acids	[35]
	<i>Phyllobacterium endophyticum</i> PEPV15	Rhizospheric bacterium	Fruit	Improved vitamin C level and yield	[36]
	<i>Paenibacillus polymyxa</i> RC05	Rhizospheric bacterium	Fruit	Improved vitamin C level and yield	[37]
	<i>Bacillus simplex</i> RC19	Rhizospheric bacterium	Fruit	Improved vitamin C level and yield	[38]
	<i>Pseudomonas</i> sp. 5Vm1K	Rhizospheric bacterium	Fruit	Improved vitamin C level	[35]
Tomato	<i>Rhizophagus irregularis</i> , <i>Funneliformis mosseae</i>	Arbuscular mycorrhizal fungus	Fruit	Increased N, P, and Cu concentrations, higher antioxidant concentrations and carotenoid contents	[39]
	<i>Pseudomonas</i> sp. 19Fv1T	Rhizospheric bacterium	Fruit	Improved vitamin C level and fructose level	[38]
	<i>Bacillus licheniformis</i>	Rhizospheric bacterium	Fruit	Improved flavonoid level	[40]
Wheat	<i>Pseudomonas jessenii</i> (R62) and <i>Pseudomonas synxantha</i> (R81) and AMF consortium (Mnat) and (Mss2)	Rhizospheric bacteria and arbuscular mycorrhizal fungi	Grain	Significantly improved selenium content	[41]
	<i>Pseudomonas</i> strain (<i>Providencia</i> sp. PW5) and <i>Anabaena</i> sp., <i>Calothrix</i> sp. and <i>Anabaena</i> sp.	Rhizospheric bacteria and arbuscular mycorrhizal fungi	Grain	increased wheat yield and mineral nutrient concentrations of P, K, Cu, Fe, Zn, and Mn	[41]
	<i>Providencia</i> sp. combined with N ₆₀ P ₆₀ K ₆₀ chemical fertilizer	Rhizospheric bacterium	Grain	Increases grain Zn accumulation to ~42 mg kg ⁻¹ compared to 31.60 mg kg ⁻¹ in control, along with a three-fold increase in the concentration of Fe.	[42]
	<i>Bacillus cereus</i> YAP6 and <i>Bacillus licheniformis</i> YAP7	Rhizospheric bacteria	Grain	Increased Se concentrations in the grain by up to 375%	[43]
	Eight AMF species (<i>Gigaspora margarita</i> , <i>Funneliformis mosseae</i> , <i>Rhizophagus irregulare</i> , <i>Glomus clarum</i> , <i>Glomus deserticola</i> , <i>Glomus monosporum</i> , <i>Glomus brasilianum</i> , <i>Glomus aggregatum</i>)	Arbuscular mycorrhizal fungus	Root	increased the uptake of P, Fe, and Zn with improved root lengths and density	[44]

Table 1. Cont.

Crop	Microbe	Type of microbe	Tissue	Impact	Reference
	<i>Glomus clarideum</i> and <i>Pseudomonas</i> sp. R8	Arbuscular mycorrhizal fungus and rhizospheric bacterium, respectively	Grain	Improved selenium level	[45]
	<i>Glomus clarideum</i> and <i>Bacillus</i> sp. R12	Arbuscular mycorrhizal fungus and rhizospheric bacterium, respectively	Grain	Improved selenium level	[45]

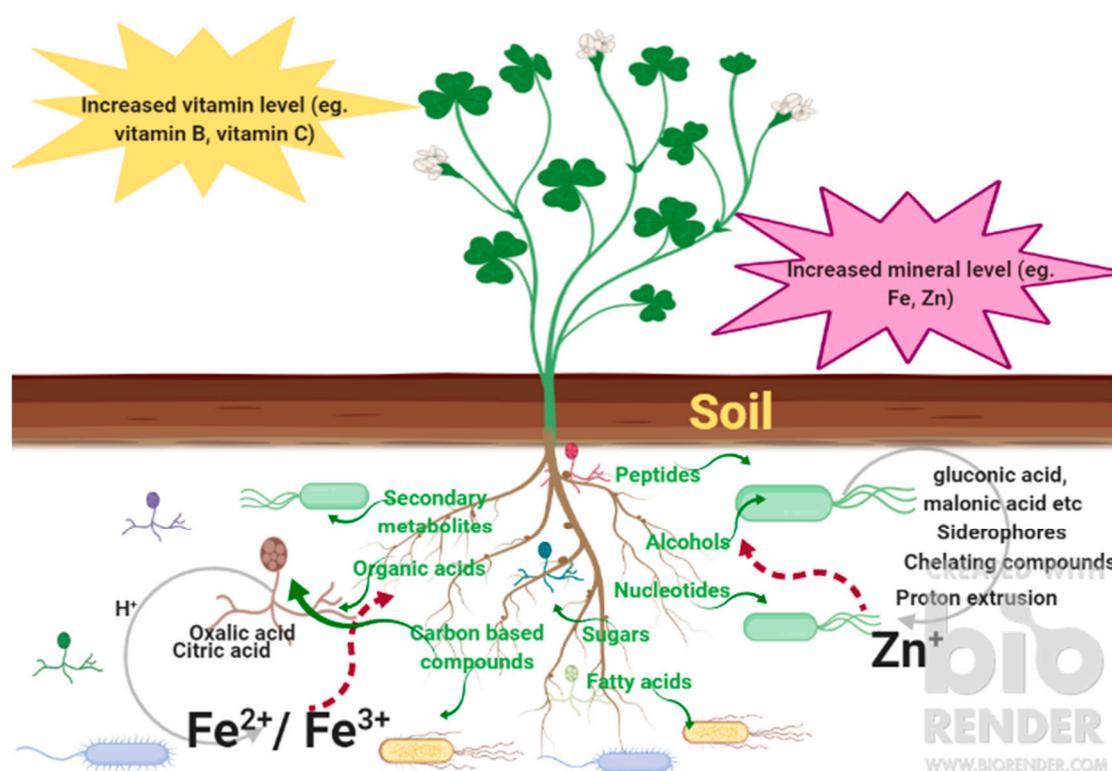


Figure 1. The complex interactions between plants and microbes in the soil bring about microbe-mediated biofortification. This figure is generated by BioRender.

2. Common Dietary Deficiencies in Minerals and Vitamins and the Corresponding Biofortification Strategies

2.1. High Iron (Fe) Acquisition Can Be Achieved through Soil Microbes

Iron (Fe) deficiency is regarded as the most common type of micronutrient deficiency in the human diet [3]. For plant growth, a wide variety of cellular processes such as chloroplast development, oxygen transport, chlorophyll biosynthesis, and mitochondrial functions are dependent upon the bioavailability of Fe in soils [46,47]. In many crops, the deficiency of Fe in soil leads to chlorosis which results in low-quality agricultural produce and poor yield [48]. Fe deficiency in plants is also closely related to the prevalence of Fe deficiency-induced anemia in humans [5].

In general, lowering the soil pH, decreasing clay contents, increasing organic matter, improving drainage, and decreasing the excessive usage of phosphatic fertilizers can improve Fe uptake in plants. The acidification of the rhizosphere surrounding the roots of legumes and the extrusion of mugineic acid-family phytosiderophores via efflux transporters in non-leguminous plants are the two innate mechanisms in plants to overcome limitations in Fe availability in soil [46,49,50]. However, several studies have indicated that these strategies alone are insufficient to prevent plants from suffering from Fe deficiency in Fe-limiting soils. Sunflower and red clover crops had decreased Fe acquisition when grown in sterile soil versus in non-sterile soil, providing evidence that soil microbial activities probably played a critical role in Fe acquisition in plants [51]. A high Fe uptake was observed in wheat, white lupin, and cucumber plants upon the inoculation of a fungus, *Trichoderma asperellum* strain T34, in the soil [52]. In fungi, a variety of Fe uptake mechanisms have been reported, including those for free Fe ions, low-affinity Fe chelates, siderophore–Fe chelates, transferrin, heme, and hemoglobin [53]. Many microorganisms synthesize and release siderophores to overcome low Fe availability in aerobic calcareous soils [54]. Over 500 siderophores have been identified based on their ligand architectures, being in the form of hydroxamate, catecholate, or hydroxycarboxylic acid [55]. The solubilizing effect

of siderophores on Fe hydroxides and their production in the rhizosphere have been proposed as a key microbial activity that benefits plants in enhancing Fe acquisition [48,51]. A high dissolution rate of insoluble Fe sources in the phenolic root exudates of Fe-deficient red clover plants was observed upon the addition of siderophores secreted by a *Pseudomonas* sp. [48]. These data indicate that Fe–siderophores may be incorporated into the roots of leguminous plants more efficiently than other Fe sources [47]. In maize, YS1 (Yellow Strip 1) has been suggested to be a Fe (III)-phytosiderophore transporter and its genetic transformation into yeast led to high iron uptake and restored normal growth [56].

A very old study indicated that each unit increase in soil pH decreases the availability of Fe up to 1000-fold [57]. The acidification of the rhizosphere via nitrification of ammonia and ammonium-based fertilizers can have an enormous positive impact on Fe availability. Soil nitrification is also catalyzed by ammonia-oxidizing prokaryotes [58]. In calcareous soils, many phosphate-solubilizing bacteria, including *Penicillium bilaji* and *Penicillium cf. fuscum*, significantly lower the soil pH in the presence of ammonium by excreting H^+ , which ultimately improves Fe solubility [59].

Besides these phosphate-solubilizing bacteria, a soil bacterium, *Bacillus subtilis* (GB03), could enhance Fe acquisition in *Arabidopsis thaliana* (*A. thaliana*) plants by activating its Fe deficiency-inducible responses, suggesting that soil micro-organisms could regulate plant Fe acquisition via signaling processes [60]. Recently the inoculation of Fe-tolerant bacterial strains, *Bacillus toyonensis* (MG430287), *Rhodococcus hoagii* (MG432495), *Lysinibacillus mangiferihumi* (MG432492), and *Lysinibacillus fusiformis* (MG430290) improved the growth and biomass of *Brassica juncea* by increasing the Fe contents in root, stem, and leaf compared to uninoculated plants [16]. Several phytohormones including auxin [61,62] nitric oxide (NO) [63], ethylene [64], cytokinin [65], and brassinosteroids [66] were also found to trigger Fe-deficiency responses in plant roots. Among these, auxin, NO and ethylene are particularly interesting due to their potential involvement in high Fe uptake by plants in the presence of soil microbes [47]. Like plants, some soil microbes also produce auxin-like compounds, such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), indole-3-ethanol, indole-3-carboxylic acid, and indole-3-aldehyde, but the mechanisms of the involvement of these compounds remain unclear [67]. However, the ratio of auxin-producing micro-organisms in soil solution incubated with the phenolic root exudates of Fe-deficient red clover is higher than in phenolic-free samples [51,61]. In addition to auxin-like compounds, soil micro-organisms also produce NO through the processes of nitrification and denitrification [68]. The soil water content determines whether nitrification or denitrification is the dominant process in the soil, which in turn controls NO generation in the rhizosphere by soil microbes. Like auxin, NO acts as a catalyst in the regulation of Fe deficiency-inducible responses. The exogenous addition of the NO donor (*S*-nitrosoglutathione) promotes the reduction of Fe and the development of root hairs and lateral roots [62,69,70]. Therefore, it is reasonable to assume that NO generation by soil micro-organisms may enhance Fe acquisition of plants grown in Fe-limiting soils [47]. Some of the soil microorganisms also produce ethylene in the rhizosphere [71]. The exogenously-applied ethylene precursor (1-aminocyclopropane-1-carboxylic acid) significantly increased the reduction of Fe and root hair development, indicating that ethylene alters the microbial activities which could ultimately improve Fe acquisition by plants in Fe-limiting soils [64,72].

In leguminous plants, nodulation is a ubiquitous process for N_2 fixation in which Fe-containing proteins play very important roles [73]. Thus, legumes have a higher need for Fe compared to non-leguminous plants. Common bean (*Phaseolus vulgaris*) under Fe limitation showed an over-accumulation of H^+ -ATPase and Fe^{2+} transport protein (IRT1) around the cortical cells of the root nodules [74]. The high Fe contents in pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) upon nodulation of the roots by *Rhizobium leguminosarum*-PR1 indicate that nodulation can systemically improve the Fe nutrition of plants [75,76]. The increased secretion of phenolic compounds from leguminous plant roots in Fe-deficient soils favors the growth of nodulating rhizobia in legumes. Thus, nodulation can be another strategy for Fe acquisition by legumes via the secretion of phenolic compound into the rhizosphere. Some of the rhizobial strains produce siderophores under Fe-deficient

conditions, which ultimately promote the synthesis of Fe-binding proteins by the plant for successful nodulation in legumes [73]. Hence, siderophore-producing rhizobia could be potential candidates for enhancing Fe acquisition, especially in legumes.

2.2. Zinc (Zn) Deficiency Can Be Eradicated Using Soil Microbes

Zinc (Zn) is one of the most vital elements for all living organisms on Earth and its deficiency can lead to severe consequences. Zn deficiency affects more than 30% of the world's human population and it causes growth retardation, impaired immune functions, skin problems, loss of appetite, hair loss, diarrhea, delayed sexual maturation, impotence, and hypogonadism in males [77,78]. In plants, Zn deficiency leads to chlorosis, retarded shoot growth, decreased leaf size, susceptibility to biotic and abiotic stresses, poor grain yield, improper pollen and root development, as well as decreased water uptake and transport [79,80]. Zn availability is a global issue, as nearly 50% of the soils used for cereal cultivation contain very little bioavailable Zn [81]. Soils with less than 0.5 mg kg⁻¹ DTPA (diethylene triamine penta-acetic acid)-extractable Zn are commonly considered to contain too little bioavailable Zn for optimal crop growth [82]. More than 50% of the agricultural soils in India and Pakistan are Zn-deficient [83]. Physical properties, such as pH, percentage of organic matter, water and clay contents, soil texture, and soil microbes, are the most critical factors that determine the bioavailability of Zn in soils [84]. Sandy soil and calcareous soil are the most Zn-deficient soil types, whereas clay soil retains more Zn due to its high water-holding capacity. Heavy rain or flooding can lead to significant loss of bioavailable Zn in soils due to leaching and changing of the soil pH. On the other hand, increasing organic matter can improve Zn solubility and availability by decreasing its binding to other soil elements. Externally applied Zn in the form of fertilizer can also be fixed with manganese (Mn) and Fe hydroxides on soil colloids [79].

Both bacteria and fungi have been found to enhance Zn solubility in the rhizosphere. The bacterial genera, *Azotobacter*, *Azospirillum*, *Bacillus*, *Gluconacetobacter*, and *Pseudomonas*, have all been identified as Zn solubilizers [85]. Arbuscular mycorrhizae and the genus *Trichoderma* are the two groups of fungi possessing Zn-solubilizing activities in soil [86]. By inoculating these microbial strains into the soil where the crops are grown, not only will the bioavailability of Zn be improved, but we can also promote a sustainable agro-ecosystem. Through the siderophore and exopolysaccharide production, nutrient solubilization, the production of organic acids and phytohormones, the mobilization of nutrients, and the fixation of atmospheric nitrogen carried out by these microbes, the availability of Zn can be raised, thus helping plants to uptake more Zn from the soil [87]. Significant increases in Zn contents in maize grains were observed when the crop was grown in soil inoculated with the bacterial strains from the genera *Azotobacter*, *Pseudomonas*, *Bacillus*, and *Azospirillum* [88,89]. In rice, following the inoculation with bacterial and endophytic strains (*Azospirillum lipoferum* [JCM-1270, ER-20], *Pseudomonas* sp. [K-1, 96-51], *Agrobacterium* sp. [Ca-18], *Sphingomonas* sp. [SaMR12], and *Enterobacter* sp. [SaCS20]), the bioavailability of Zn in the soil was significantly enhanced, resulting in higher levels of Zn in the grains and the yields were also improved [29,90,91]. In soybean and wheat, an increase in the mobilization of Zn and its concentrations in the edible portions were observed following the inoculation of the soil with *Bacillus aryabhatai* strains (MDSR7, MDSR11, and MDSR14) [92]. The two newly identified bacterial strains (FA-9 and FA-11) from the wheat rhizosphere, belonging to the species *Pseudomonas aeruginosa* and *Enterobacter* sp., possess a great potential to solubilize Zn from Zn carbonate in a liquid medium, along with carrying out various rhizospheric activities such as the production of exo-polysaccharides and siderophores, P solubilization, ACC (1-aminocyclopropane-1-carboxylic acid) deaminase, and antifungal activities [93]. Particularly in wheat, the inoculation of *Providencia* sp. (PW5), *Anabaena* sp. (CW1), *Calothrix* sp. (CW2), and *Anabaena* sp. (CW3) did not only increase the Zn contents in grains but also improved the protein contents and other micronutrients such as Fe, Cu, and Mn [42]. Recently, the discovery of Zn nanoparticles from the cell-free culture filtrates of *Pseudomonas*, *Bacillus*, and *Azospirillum* strains suggests that these microbes may solubilize Zn by producing nanoparticles to mobilize nutrients in the rhizosphere [94]. Eight strains from the genera

Curtobacterium, *Plantibacter*, *Pseudomonas*, *Stenotrophomonas*, and *Streptomyces* have been successfully identified from the wheat rhizosphere to be highly efficient in Zn solubilization as well as siderophore production [95]. A high level of mobilization of Cu and Zn was observed in spinach (*Spinacia oleracea* L.) and tomato (*Lycopersicon esculentum* L.), which indicates that the release of organic ligands improves the solubility of trace elements in soil. On the other hand, the insufficient release of citrate and malate compounds by rice plants growing in Zn-deficient soil shows that the effectiveness of a specific exudate is likely to vary in different soil types [96,97]. The success of these inoculated strains depends upon their ability to colonize, survive and mobilize trace elements in the rhizosphere under natural conditions. It is necessary to understand the molecular mechanisms behind Zn solubilization by these bacteria before planning to use them as inoculants. However, despite various potential applications of Zn-solubilizing bacteria, few studies have focused in detail on the underlying mechanisms of solubilization.

Zn-solubilizing bacteria improve Zn bioavailability in soil through various mechanisms of action [83]. In general, reducing the soil pH is the key to unlocking many micro- and macro-nutrients in the rhizosphere. A one-unit decrease in soil pH can improve Zn availability 100 times [98]. These bacteria achieve this by the release of organic acids and thus protons into the rhizosphere [99]. For example, the secretion of 2-ketogluconic acid and gluconic acid by *Pseudomonas fluorescens* solubilizes Zn phosphate in the liquid culture [100]. Similarly, a change in pH of the liquid culture was observed upon the co-inoculation of *Pseudomonas* and *Bacillus* species, which solubilized the Zn-containing complexes in that culture [101]. It is important to mention that Zn-solubilizing bacteria do not rely on only one strategy to solubilize the trace elements. Instead, the mechanism employed changes depending on the situation. For example, the presence of glucose in the liquid medium stimulated organic acid (such as gluconic, malonic, and oxalic acid) production by eight Zn-solubilizing bacterial strains [95], leading to the acidification of the medium and subsequent ZnO solubilization. In contrast, in the absence of glucose, ZnO dissolution resulted from proton extrusion (e.g., via ammonia consumption by *Plantibacter* strains) and complexation processes (e.g., complexation with glutamic acid in the culture of *Curtobacterium* sp.). Bacteria are not the only organisms that can secrete organic acids, but some of the arbuscular mycorrhizal fungi could also solubilize Zn phosphate by secreting organic acids into the rhizosphere as well [102]. A decrease in rhizospheric soil pH upon the inoculation of arbuscular mycorrhizae contributed to the release of Zn from the mineral fraction [103]. However, the degree of reduction in soil pH depends upon many factors such as the type of soil, soil texture, geographic location, and its associated soil microbial communities. The high reactivity and low persistency of Zn in soil result in the formation of Zn-metal complexes that hinders its availability to plants. Naturally existing and synthetic Zn-chelating compounds also have the potential to enhance Zn bioavailability in the rhizosphere [104]. Chelation is also a dominant mechanism used by many Zn-solubilizing bacteria, such as *Pseudomonas monteilii*, *Microbacterium saperdae*, and *Enterobacter cancerogenus*, to make chelating metallophores which increase the concentration of water-soluble Zn in the soil for uptake by plants [105]. A biofertilizer containing *Pseudomonas* sp. (96-51), *Azospirillum lipoferum* (JCM-1270, ER-20), and *Agrobacterium* sp. (Ca-18) increased Zn bioavailability for longer periods in the soil by releasing ethylenediamine-tetraacetic acid (EDTA) as the chelating agent [91]. Zn is an immobile element in the soil and expanding the root surface area could be another strategy to increase Zn availability. The association of mycorrhizal fungi with roots can increase the surface area of the root, helping plants to take up more Zn from the surrounding soil. An increase of up to 4% in Zn in the grains was observed in plants as a result of the association with mycorrhizal fungi [103].

2.3. Vitamins Are Popular Targets of Biofortification

Golden Rice has been a golden example of biofortification of provitamin A. In 2016, scientists who developed the orange-fleshed sweet potato to combat vitamin A deficiency were recognized by the World Food Prize [106]. Current methods of improving the provitamin A contents in crops include breeding and transgenic approaches [107]. Taking Golden Rice as an example, having a gene from daffodil (*Narcissus pseudonarcissus*) and a bacterial gene from *Erwinia uredovora* introduced into rice

through transgenic approaches allows Golden Rice to start synthesizing β -carotene [108]. Because many crops cannot produce β -carotene natively, the immense capital investment and technical know-how required to produce transgenics capable of synthesizing β -carotene in each of these crops severely limit the scope of this approach to biofortification. The arduous approval process for transgenic crops to be sold on the market is also a significant hurdle. It took 18 years after the initial publication of the research on Golden Rice before it was finally approved by the U.S. Food and Drug Administration in 2018 [109]. Hence investigating ways of biofortification of provitamin A contents in crops using microbes remains a wide-open and much-needed area for research.

The B vitamins and vitamin C can be synthesized in numerous food crops. The pathways of B vitamins and vitamin C biosynthesis are well-studied [110,111]. The enrichment of vitamins B and C contents in food crops has been a focus of biofortification. It has been shown that plants can take up vitamins B₁ (thiamine) and B₁₂ (cobalamin) from soil [112]. Although plants can synthesize vitamin B₁, the contents of vitamin B₁ in staple crops are generally low. Therefore, the deficiency in vitamin B₁ is a common problem in areas where human diets consist overwhelmingly of staple crops. There have been attempts to improve the vitamin B₁ levels in crops using the transgenic approach. However, it is only recently that the challenge has been overcome [113,114]. The first example of vitamin B₁ biofortification in staple crops using transgenics was reported in 2016 [115]. The overexpression of 4-methyl-5- β -hydroxyethylthiazole phosphate synthase and 4-amino-2-methyl-5-hydroxymethylpyrimidine phosphate synthase, which are involved in the synthesis of thiazole and pyrimidine, respectively, led to the increase in the thiamine content of unpolished rice grains [115]. As early as the 1950s, it was found that fodder plants irrigated with sewage water had higher vitamin B₁ contents than those irrigated with clean water [116]. Later, it was found that spinach grown organically had a higher vitamin B₁ content than that grown by conventional methods [117]. Similarly, for green gram and wheat, it was found that the application of cow dung or cane-bagasse in the fields could improve the vitamin B₁ contents of the plants [118]. These findings hinted at the role of microbes in improving the vitamin B₁ levels in plants. In 2017, a detailed study on the microbe-mediated biofortification of vitamin B₁ was first to report on the improvement of thiamine biosynthesis in oil palm seedlings by the introduction of *Hendersonia toruloidea*, an endophytic fungus that colonizes the cortical cells of the oil palm root [21,22]. Oil palms inoculated with *H. toruloidea* showed increased expressions of key thiamine biosynthesis genes including *THI4*, *THIC*, *TH1*, and *TPK* for 30 days after the inoculation until they were downregulated [21,22]. Consequently *H. toruloidea*-inoculated oil palms showed improved vitamin B₁ accumulation in the leaves [21,22].

Among the eight B vitamins, vitamin B₁₂ is unique as it is synthesized by microorganisms but not by plants. Barley and spinach treated with pure vitamin B₁₂ or cow dung showed increased vitamin B₁₂ levels in the seeds and leaves, respectively [112]. The uptake of vitamin B₁₂ by soybean roots in a vitamin B₁₂-supplemented nutrient solution was also reported [119]. As early as the 1950s, it was found that the vitamin B₁₂ content in turnip greens was geography-dependent [120]. It was then speculated that vitamin B₁₂ might have originated from microbes [121]. By adding cow dung to soil to increase the vitamin B₁₂ level in spinach leaves, the microbial origin of vitamin B₁₂ was confirmed [119]. Due to the lack of vitamin B₁₂ synthesis genes, crops cannot innately produce vitamin B₁₂. However, as demonstrated previously, plants can take up vitamin B₁₂ from the surrounding soil and transport it to the edible parts. Therefore, the application of vitamin B₁₂-synthesizing bacteria to the soil for growing crops could be a way to improve the vitamin B₁₂ contents of crops. Compared to the current practice of applying vitamin B₁₂-containing chemical fertilizers, this could be a “greener” alternative. Although many processed crop products, such as wheat flour, are routinely fortified with vitamins during production [122], fortifying growing crops with vitamin B₁₂ using the microbe-mediated approach has, up to now, been rarely done.

Fruits are major sources of vitamin C in the human diet. As a result, fruits have been major targets of biofortification with vitamin C [123]. Common approaches of vitamin C biofortification include the application of fertilizers, the breeding of elite germplasms, and metabolic engineering [123,124].

For example, the overexpression of *SIGME1* or *SIGME2* enhanced ascorbate accumulations in the leaves and ripe fruits of tomato [125]. Besides the improved ascorbate accumulation, the transgenic plants were also found to have improved tolerance to oxidative stress, cold stress, and salt stress [125]. Both bacteria and fungi were also found to enhance vitamin C levels when inoculated into/onto the plants [126,127]. Strawberry and tomato have been the popular targets for vitamin C biofortification using microbe-mediated approaches [126]. The improvement of vitamin C content is usually accompanied by the improvement of other agronomic traits. For instance, the inoculation of *Phyllobacterium endophyticum* strain PEPV15, *Paenibacillus polymyxa* RC05 isolated from barley, and *Bacillus simplex* RC19 isolated from wild raspberry improved the vitamin C contents in the fruits of strawberries and also the yields [36,37], and the inoculation of *Pseudomonas* sp. 19Fv1T to the soil used to grow tomato plants enhanced both the vitamin C and the fructose levels of the fruit [38]. The inoculation of *Pseudomonas* sp. 5Vm1K to the media used to grow strawberry plants improved the vitamin C, glucose, and fructose levels in the fruit [35]. The inoculation of *Glomus fasciculatum* or a commercial inoculum consisting of *Glomus intraradices* (Schenck and Smith) and *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe to the growth medium of lettuce improved the ascorbate level as well as the fresh weight of the leaves [20]. Inoculation of blue-green algae has been used as a measure to improve crop yield. However, it was found that the inoculation of blue-green algae, as in a mixture of *Tolypothrix tenuis*, *Aulosira fertilissima*, *Nostoc* sp. *Anabaena* sp., and *Plectonema boryanum*, to tomato improved the yield but not the vitamin C level in the fruit [128]. In some cases, the inoculation of microbes negatively affected the fruit vitamin C content [38,129]. For example, the inoculation of a mixture of plant growth-promoting rhizobacteria (PGPR), consisting of *Pseudomonas* BA-8 (a biological control agent), *Bacillus* OSU-142 (N₂-fixing), and *Bacillus* M-3 (N₂-fixing and phosphate-solubilizing), had a negative impact on the fruit vitamin C level of strawberry [129]. Therefore, microbe-mediated biofortification of vitamin C has the potential to bring forth great benefits but more studies are required to fully understand the underlying mechanisms in order to reap the full benefit of this approach.

3. Microbes Enhance the Level of Beneficial Secondary Metabolites

In under-developed regions, there is a need to fortify crops to ensure that sufficient essential micronutrients can be obtained from the diet. In wealthy societies, biofortification of crops could be a way to let humans obtain non-essential health supplements. There are limited examples of flavonoid and saponin biofortification. Detailed mechanisms of how the microbes mediate the biofortification of flavonoids and saponins are largely unknown. Nevertheless, the following examples illustrate the potential of microbes to be used for fortifying health supplements besides the aforementioned micronutrients.

3.1. Flavonoids

Flavonoids are a large group of secondary metabolites characterized by a 15-carbon skeleton and a polyphenol molecular structure [130]. The molecular structure of flavonoids contributes to their antioxidizing activities and hence the benefits to human health [131]. Unlike the above-discussed micronutrients which are regarded as essential, flavonoids are not considered essential nutrients, but the health benefits of flavonoids have widely drawn the public's attention in recent years [130]. Breeding and metabolic engineering are major strategies of flavonoid biofortification [132]. For example, the expressions of both *LC* and *C1*, which are transcription factors from maize, enhanced the levels of flavonols (a major group of flavonoids) in the flesh of the tomato fruit, which does not innately produce flavonoids [133]. Some reports have provided direct evidence of using microbe-mediated biofortification to improve flavonoid levels. It was found that the inoculation of *Pseudomonas fluorescens* N21.4 to blackberry plants increased the flavonoid levels of the fruit [14,15], probably by upregulating the expressions of several flavonoid biosynthesis genes [15]. Additionally, the inoculation of *Bacillus licheniformis* to tomato plants, together with 75% of the recommended dosage for nitrogen fertilizer, improved the flavonoid levels in the fruit [40]. While it has been a concern that flavonoids are heat-labile

and could be destroyed during the cooking process [134], the flavonoid-fortified tomato fruit, which can be, and often are, eaten raw, would be a good dietary source for these antioxidants.

3.2. Saponins

Saponins are a group of bitter-tasting compounds regarded as the active compounds responsible for the health benefits of foods such as soybean and garlic, as well as plants used in herbal medicine [135]. Their anticancer activities [136] and cholesterol-lowering abilities [136] have been suggested. Plants are the major sources of saponins in the human diet [135]. The enrichment in saponin levels in crops could be beneficial for human health. Not all plants are rich in saponins. However, it has been shown that transgenic methods could be used to improve the saponin levels in crops such as rice, which innately does not have high levels of saponins. The overexpression of a β -*amyrin synthase* gene from *Panax japonicus* led to the accumulation of ginsenoside, an oleanane-type saponin that is the bioactive compound in ginseng, in rice seeds [137]. Endophytic mycorrhizal fungi have been known to synthesize saponins. A few *Fusarium* spp. isolated from *Panax ginseng* were found to be able to produce ginsenosides [138]. Safed musli (*Chlorophytum borivillianum*) is a medicinal plant. It was found that the inoculation of endophytic fungi, either *Glomus fasciculatum*, *Glomus intraradices*, or *Glomus mosseae*, enhanced the saponin contents in the tubers of this plant as well as enhancing the biomass of both roots and shoots [31]. In another study on safed musli, both endophytic mycorrhizal fungus and rhizospheric bacterium were also found to have beneficial effects on its saponin levels. When the micropropagated plants of safed musli were inoculated with the endophytic mycorrhizal fungus, *Piriformospora indica*, it was found that the saponin contents were increased [32]. This beneficial effect was further enhanced when the plants were co-inoculated with both *P. indica* and the growth-promoting bacterium, *Pseudomonas fluorescens* [32]. The co-inoculation of *P. indica* and *P. fluorescens* also led to the increased contents of micronutrients including Fe, Zn, and Mn [32].

4. Endophytes as the Emerging Participant of Microbe-Mediated Biofortification

Literally, “endophyte” means “in the plant” [139]. Endophytes are generally known as non-pathogenic microbes that colonize inside plant tissues including roots [140]. For example, as mentioned above, *H. toruloidea*, is found to be an endophytic fungus of oil palm that colonizes the cortical cells of the oil palm root [21,22]. The beneficial effects of root endophytes on plant growth has been extensively discussed [139]. Besides plant growth, there is emerging evidence showing the involvement of endophytic fungi in biofortification. The symbiotic mechanism involves extensive interactions between plants and endophytes. Plants excrete metabolites to initiate the symbiosis with these fungi. For mycorrhizal symbiosis, the transport of fatty acids (linoleic, oleic, palmitic, and/or stearic acid) by ABCG (ATP-binding cassette transporter subfamily G) proteins is essential because mycorrhizal fungi depend on their hosts for certain fatty acids [141,142]. One ABCG member, STR (stunted arbuscule), was previously shown to be required for mycorrhization [143]. Secondary metabolites such as coumarins (e.g., esculetin, esculin, scopoletin, scopolin, 4-methylumbelliferone), sterols (e.g., campesterol, cholesterol, sitosterol, stigmasterol), hormones, and glucosinolates are omnipresent in root exudates, and ABC transporters are potentially responsible for their transport into the rhizosphere. Exudates of *A. thaliana abcg37/pdr9* mutant lines were found to be deficient in several phenylpropanoids [144].

Endophytes can enhance the accumulation of nutrients including Fe, Zn, B vitamins, vitamin C, flavonoids, and saponins. In addition, endophytes can also enhance the accumulation of nitrogen and minerals in crops. Since the adverse effects of nitrogen fertilizer on the environment has been well known [145], microbe-mediated biofortification could be a “greener” alternative to provide the crops with nitrogen. Legumes are famous for its ability to form nitrogen-fixing nodules with rhizobia. The rhizobium-mediated nitrogen fixation in the root nodules of legumes has been extensively reviewed [146,147]. For non-legume crops, it was reported that in one case, an increase in plant nitrogen acquisition was found to be dependent upon the synergies between mycorrhizal fungi and

soil microbial communities [148]. Specifically, it has been reported that endophytic microbes can translocate nitrogen from insects to the host plant. By inoculating the endophytic fungus *Metarhizium rebertsii* into haricot bean and switchgrass, it was shown that *M. rebertsii* translocated nitrogen from waxmoth, the prey of *M. rebertsii*, to the host plant [149]. This endophyte-mediated non-soil origin of nitrogen for plants further expands the potential of microbe-mediated biofortification.

Apart from nitrogen, endophytes were found to improve the level of other minerals in crops. This helps reduce the use of chemical fertilizers. It was found that the inoculation of *Bacillus subtilis* DS-178 and *Arthrobacter* sp. SD-179 improved the level of Zn in the grains of wheat fertilized with the recommended dosage of an NPK fertilizer [150]. Besides, it was found that *Enterobacter* sp. ScCS20 and *Sphingomonas* sp. SaMR12, which were isolated from the Zn-hyper-accumulator *Sedum alfredii*, were able to colonize rice roots, increasing the bioavailability of Zn in the rhizospheric soil and enhancing the level of Zn in the rice grains [29]. When wheat plants were co-inoculated with *Glomus clarideum* and either *Pseudomonas* sp. R8 or *Bacillus* sp. R12, the selenium level in the grains was improved compared to inoculating with either of the bacteria alone [45]. Besides, mycorrhizae also excrete H^+ and low-molecular weight organic chelating compounds, such as citric acid, oxalic acid, and siderophores, to solubilize Fe in soil [151]. Recently, mycorrhizal inoculation in wheat roots was reported to have increased the uptake of P, Fe, and Zn by the plant, along with greater root length and density [44]. In maize, the symbiosis of mycorrhiza alters the expression patterns of three key iron homeostasis genes in sulfur-deprived plants, which indicates sulfur works as a signaling molecule for Fe homeostasis [152,153]. The recent growing evidence on the beneficial effects of endophytes suggests that the current knowledge on endophytes could be only the tip of the iceberg.

5. The Mechanisms of Microbe-Mediated Biofortification

To summarize the above discussion, the mechanisms of how the nutrient levels in plants are improved by microbe-mediated biofortification can be categorized into three types.

5.1. By Improving the Availability of Nutrients to Be Absorbed by Plants

A lot of nutrients, such as minerals, are already present in the soil. However, the minerals may not be in the chemical forms that could be readily taken up by plants. One of the examples is Fe ions, which are usually present in hydroxide form in soil. Some microbes solubilize the hydroxide to free the Fe ions for the plants to import, whereas some other microbes, including mycorrhizal fungi [151] and growth-promoting bacteria [48], excrete small molecules (siderophores) to solubilize Fe in soil [151]. Besides mediating the availability of nutrients in soil, endophytic fungi have also been found to be able to mediate the transfer of nitrogen sources from insects to plants [149]. Besides, *Azotobacter*, *Azospirillum*, *Bacillus*, *Gluconacetobacter*, and *Pseudomonas*, all genera of growth-promoting bacteria [85], and *Trichoderma*, a genus of fungi, have all been reported to exhibit Zn-solubilizing activities in the soil [86].

5.2. By Directly Synthesizing and Releasing Micronutrients Either in the Soil or as Endophytes

Microbes are able to synthesize some important nutrients. For example, vitamin B₁₂ is synthesized by microbes but not by plants. Several endophytic fungi are able to synthesize saponins such as ginsenosides [138].

5.3. By Inducing the Plants to Synthesize the Micronutrients

In some cases, it was found that the inoculation of microbes induced the expression of micronutrient synthesis-related genes. For example, the inoculation of *H. toruloidea* into oil palms led to the higher expressions of *THI4*, *THIC*, *TH1*, and *TPK*, which are related to thiamine synthesis [21,22]. In blackberry, it was shown that the inoculation of *P. fluorescens* N21.4 led to the increased expressions of several flavonoid biosynthesis genes [15].

6. How Root Exudates Help to Shape the Rhizobiome

Plant health in natural environments relies on its interactions with a complicated and diverse group of macro- and micro-organisms. Plants can host up to 1000 distinct microbial species by supplying them with carbon sources via root exudation in the rhizosphere [154]. Twenty percent of the carbon fixed by photosynthesis is released into the rhizosphere, which works as a nutrient-clearing house for promoting successful plant–microbe interactions [155]. In turn, plant-associated microbiota improve plant growth by enhancing the bioavailability of nutrients such as N, P, and K to plants, as well as protecting plants against phytopathogens [156]. In addition to providing a source of carbon, plant roots also supply other metabolites that are more beneficial to some microbes than others [157]. Root-derived exudates mainly consist of sugars, alcohols, phosphates, amino acids, organic acids, nucleotides, peptides, fatty acids, and secondary metabolites [158]. Sugars constitute a significant proportion of exudates, and are a major source of carbon for microbes [159]. *Sweet* (sugars-will-eventually-be-exported transporters)-mutant plants showed higher sugar exports from roots than wild-type plants [160]. Until now, no sugar transporters directly involved in exporting sugars into the rhizosphere have been identified. The presence of higher sucrose concentrations around young and permeable root tissues, compared to older and less permeable ones, indicates that a passive sugar efflux mechanism may be involved in sugar transport in the rhizosphere [161]. A higher rate of release of glutamate, glucose, ribitol, citrate, of *c*-aminobutyric acid, and carbohydrates from Fe- and P-deficient plants suggests that these root exudates may be intended for attracting more microbes that can help with the release of nutrients from the soil. K-starved plants released less sugar, glycerol, ribitol, fructose, and maltose, while, under nitrogen deficiency, lower amounts of amino acids were found in root exudates [162]. Sugar alcohols including inositol, myo-inositol, threitol, xylitol, erythritol, and ribitol are also detected in exudates but their modes of export by plants are not well understood [158]. The involvement of sugar phosphates in plastid-localized sugar–phosphate transport and intracellular carbohydrate metabolism have been reported in several plant species [163]. Although sugar phosphates have been detected in exudates, their mechanisms of import and export remain unclear.

Amino acids make up an important fraction of exudates characteristic of the early stages of root colonization and are detected by microbial chemoreceptors [164]. The higher tryptophan exudation from older roots than younger ones [161] indicates that transporter proteins are involved in exudation. The variation in the concentrations of amino acids in root exudates and root extracts are not the same for all the amino acids, suggesting that at least some amino acids are selectively transported [165]. The secreted amino acids from the roots of ryegrass lowered the soil pH and increased the availability of free Zn in the soil [166]. Organic acids, such as succinic, malic, tartaric, lactic, formic, butyric, acetic, propionic, gluconic, oxalic, citric, pyruvic, malonic, α -ketoglutaric, fumaric, trans-aconitic, aspartic, benzoic, and glyceric acid, make up a big portion of exudates and serve as microbial nutrients [158]. Aluminum-activated malate transporters (ALMTs) and the multidrug and toxic compound extrusion (MATE) family of transporters responsible for exporting malate and citrate are among the few well-understood examples of transporters involved in organic acid exudation [158]. These root exudates also improve the cation exchange capacity in the soil by providing ion exchange sites for Zn and other mineral elements [167]. Root exudates also include nucleotides (adenosine, guanosine, cytidine, thymine) and oligopeptides which are also actively imported by secondary transporters, but the mechanisms of their exudation remain unclear [168,169].

It is also important to understand here that plant roots also secrete antimicrobial peptides that hinder microbial colonization as a result of immune responses, of which only a selected group of microbes are tolerant [170]. In maize, a change in the amount of exuded amino acids, sugars, and organic acids under potassium-deficient conditions indicates that exudations are also modulated by abiotic stresses [171]. Other examples include the increased exudation of coumarin and oligolignol by P-deficient *A. thaliana* plants, and increased organic acid exudation by *Populus tremula* under heavy metal stress, and increased phyto siderophore exudation by Zn-deficient wheat [172,173]. A report

recently showed that exuded coumarin in response to Fe starvation reshaped the microbiome and could also protect the *A. thaliana* against the pathogenic fungi, *Fusarium oxysporum* and *Verticillium dahliae* [174]. In *A. thaliana*, the assembly of the root microbiome is highly influenced by MYB72 and BGLU42, which are involved in the biosynthesis and exudation of scopoletin (a coumarin) into the rhizosphere. Furthermore, this process is triggered by beneficial bacteria. Some of the beneficial root microbes also stimulate a defense mechanism in the plant known as induced systemic resistance against diseases caused by foliar pathogens [175]. Differential exudation is a plausible mechanism by which plants could modulate their interactions with microbes, as illustrated by the correlations between exudation patterns and rhizobiome variations reported for eight *A. thaliana* accessions [176].

The effect of plant genotype on rhizobiome composition was found to be similar between *A. thaliana* and barley (*Hordeum vulgare*) grown in the same experimental conditions with respect to the relative abundances and distributions of taxonomic groups of the microbes [177]. Intraspecific plant variations can prompt changes in the surrounding environment (soil conditions) and the compositions of underground microbiota, potentially inducing plant–soil feedback responses [178]. Interestingly, some species, such as maize and birdsfoot trefoil (*Lotus japonicus*), assembled a distinct rhizobiome, while other species, such as *A. thaliana* and rice, assembled a rhizobiome-like bulk soil [158]. The integrity of the rhizosphere differs according to the developmental stage of the plant [179,180]. Similarly, root exudation and microbial communities change with the age of the plant [179]. Various developmental phases of *A. thaliana* can be correlated to distinct rhizobiomes and similar results were identified in *Avena fatua* [179,181] and in rice [182]. Moreover, crop duration was also discussed as a hypothesis for successive microbiomes [182]. Genetic variations in crops have an impact on root structure, composition, and exudates, and many studies have attempted to determine the extent of plant genetics as a determinant on its associated root microbiomes [183]. In general, these studies have shown that plant genotype affects the root microbiome, but it does not make as much a difference as soil composition does on the microbiome [170].

Previous studies have shown that the radial proximity and lateral position of microbes relative to the roots define the microbiome complexity and its composition [181,184,185]. The root structural components, which include root tips, root elongation and maturation zones, and lateral root number, are also responsible for determining a distinct microbial community that is different from another [184–187]. The root tip is the first component of the root that comes into direct contact with the microbiome. It produces border cells and mucilage, essential for interactions between plant and microbes [158]. The longevity of the border cells in soil is different for different plants. Individual border cells in maize remained alive in soil for months, probably due to the presence of starch deposits [188], whereas border-like *A. thaliana* cells survived for only two weeks [189]. Cluster roots formed under low pH are dominated by *Burkholderia* species that metabolize citrate and oxalate, while in nutrient-poor soils the roots exude high amounts of organic acids and protons to solubilize phosphates [190,191]. In addition to organic acid exudation, mature cluster roots also exude isoflavonoids and fungal cell wall-degrading enzymes, resulting in reduced bacterial abundance and fungal sporulation [192]. A recent review paper briefly addressed how root morphology shapes rhizobiomes in the rhizosphere [158].

Interactions between plants and microbes are not only dependent on root morphology and exudates, but they are also shaped by microbe–microbe interactions. Several studies have examined whether the diversity of plant exudates correlates with microbial diversity. Some studies discovered that higher plant exudate diversity was associated with higher microbial diversity [193,194], and the addition of a diverse exudate mix to plant monocultures increased microbial diversity [195]. In conclusion, the influence of environmental factors on microbial diversity was greater than that on plant diversity, and high plant diversity appears to promote a diverse microbial community [194]. However, it is still unclear whether microbe–microbe interactions are predominantly positive or negative within the rhizosphere [158], but some network analyses reported predominantly positive intra-kingdom interactions [180,196].

7. Conclusions

Threats to global food security and the current and future impacts of hidden hunger on modern life have been highlighted by many authors to date [6,197]. Undoubtedly modern breeding tools such as genetic modifications and metabolic engineering can combat hidden hunger effectively but they are often time-consuming, expensive, and encounter many regulatory obstacles regarding GMOs. As alternatives, rhizospheric and endophytic microbes can serve as cost-effective and sustainable solutions to overcoming hidden hunger. Microbes can be easily applied to the growing crops for biofortification purposes. Yet, the easily-applied microbe-mediated fortification involves complex interactions between plants and microbes in the soil. Such interactions are part of the dynamic process in which plants and microbes regulate one another's growth. Using examples from the literature, here we have highlighted the great potential for plant growth-promoting bacteria and mycorrhizal fungi to be applied as a sustainable and affordable alternative for crop biofortification.

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