***Review Article***

**Microbial Synergy for Zinc and Iron Biofortification: Enhancing Crop Nutrition Through Plant-Microbe Interactions**

**ABSTRACT**

Zinc (Zn) and iron (Fe) are essential micronutrients crucial for numerous physiological and metabolic functions in plants and humans. Their prevalent deficiencies in soils is a pervasive question escort to abridged crop productivity and throw in notably to malnutrition, on global scale, principally in mounting regions where staple crops form the bulk of the diet. To address the dietary micronutrient deficiencies, a range of interventions has been implemented, with biofortification standing out as a sustainable and economically viable long-term solution. Biofortification may be accomplished via agronomic, conventional breeding, transgenic, and, more recently, microbe-assisted modalities. This review delineates the pivotal role of plant growth-promoting microorganisms (PGPMs)—integral agents in biogeochemical nutrient cycling—in augmenting the accumulation of zinc and iron in crop plants. These microbes mediate nutrient acquisition through a intricate mechanisms, including the biosynthesis of organic acids, phytohormones, and siderophores; upregulation of metal transporter gene expression; morphological and anatomical modulation of root architecture; and enhancement of nutrient bioavailability via the solubilization of zinc, phosphorus, and potassium. Furthermore, microbial attributes such as nitrogen fixation, exopolysaccharide (EPS) secretion, biofilm formation, and antagonistic activity against phytopathogens collectively strengthen plant health and facilitate micronutrient assimilation. Elucidating and strategically exploiting these multifaceted microbial functionalities lead to the development of environmentally benign and sustainable biofortification paradigms and advance global food and nutritional security.

Keywords: Microbes, Biofortification, Plant growth, Zinc and Iron

1. **INTRODUCTION**

Micronutrient deficiencies, commonly referred to as "hidden hunger," affect nearly one-third of the global population, impacting approximately two billion individual’s worldwide (**Bailey et al., 2015**). This widespread issue is predominantly driven by insufficient intake of key nutrients like iron, zinc, vitamin A, and folate, often stemming from diets dominated by low-cost staple crops with minimal diversity (**Van et al., 2020**). In India, soil nutrient deficiencies auxiliary worsen this trouble, with 19.2% of soils deficient plenty iron and 51.2% deficient in zinc (**Shukla et al., 2021**). These deficiencies considerably influence human wellbeing, agricultural productivity, and economic advancement.

Iron (Fe) and zinc (Zn) are requisite for both plant and human health. Zinc plays a decisive role in plants by supporting photosynthesis, carbohydrate synthesis, auxin metabolism, and protein production, while also ensuring pollen viability and plasma membrane integrity (**Rashid, 1994; Alloway, 2008**). Zinc deficiency in plants hampers these physiological processes, leading to growth stunting, leaf chlorosis, and reduced grain quality (**Mumtaz et al., 2017**). Similarly, iron is vital for chlorophyll synthesis, photosynthesis, and respiration in plants, and its deficiency causes interveinal chlorosis and decreased crop yield (**Briat et al., 2015**). In humans, these micronutrients are equally essential. Zinc supports immunity, growth, and wound healing, while iron is integral to oxygen transport, DNA synthesis, and energy metabolism. Deficiencies in these nutrients lead to growth retardation, anemia, and immune dysfunction (**Malik and Maqbool, 2020; Kumar et al., 2022**).

Addressing these nutritional challenges demands innovative strategies. Among approaches like supplementation, fortification, and dietary diversification, biofortification has emerged as a sustainable and cost-effective solution. This technique involves enriching crops with essential nutrients through genetic engineering, conventional breeding, agronomic interventions, and microbial assistance (**Naik et al., 2024; Jinjala et al., 2024**). Microbe-mediated biofortification, in particular, offers an eco-friendly approach by utilizing rhizospheric and endophytic microorganisms to enhance nutrient uptake in plants. These microbes improve soil fertility, mobilize nutrients like Fe and Zn, and promote plant growth through mechanisms such as siderophore production, organic acid secretion, and pH modulation (**Chen et al., 2014**). Recent studies demonstrate the potential of microbial inoculants in enhancing crop nutrition. For instance, zinc-solubilizing bacteria increase zinc uptake in soybeans, while siderophore-producing bacteria enhance iron content in wheat grains (**Sharma et al., 2013; Sun et al., 2021**). Such approaches not only boost crop yield and nutritional quality but also address global malnutrition by ensuring nutrient-rich food availability. This review underscores the significance of microbe-mediated biofortification as a promising strategy to mitigate zinc and iron deficiencies, contributing to improved agricultural productivity and nutritional security.

1. **STATUS OF IRON (FE) AND ZINC (ZN) IN SOIL**

The Green Revolution, while addressing food demands, has led to a decline in soil micronutrient content, particularly in India (**Pingali, 2023; Kaur et al., 2021**). Essential micronutrients like zinc, iron, manganese, copper, and nickel are crucial for crop growth (**Barker and Pilbeam, 2015**). Indian soils show significant deficiencies: 49% in zinc, 12% in iron, and varying levels in other micronutrients (**Singh, 2008**). This scarcity affects crop yields and food security, driven by factors such as poor organic carbon, low clay levels, intensive cropping, and the use of nutrient-deficient fertilizers (**Anand et al., 2019**).

**3. CAUSES OF MICRONUTRIENT DEFICIENCIES ADDRESSING CLIMATE CHANGE**

Iron (Fe) and zinc (Zn) are crucial for human nutrition but are often deficient in diets worldwide (**Shekari et al., 2015**). Environmental factors contributing to plant micronutrient deficiencies include inadequate light, varying temperatures, heavy rainfall, increased CO2 levels, flooding, pathogen infections, soil composition, and antagonistic effects of specific micronutrients (**Giri et al., 2017; Neenu and Ramesh 2020**). Micronutrient deficiencies are prevalent in neutral to alkaline soils and anaerobic conditions in arid regions (**Kalra et al., 2020**). Soil pH, influenced by temperature and precipitation, affects crop growth, microbial activity, and nutrient availability (**Zhang et al., 2019**). Reduced humidity and low temperatures decrease root functioning and nutrient diffusion, while cold, damp soils inhibit Fe mobilization (**Kumar et al., 2016**). Staples like rice, wheat, and maize are low in Zn and Fe, which are further reduced by processing methods like milling and polishing (**Shariatipour and Heidari, 2020**). Climate change also heightens crop susceptibility to diseases, further threatening food security (**Dong and Ronald, 2019**). This issue extends to industrialized nations, where micronutrient-poor diets are increasingly prevalent (**Allen et al., 2006**). Addressing these challenges is critical for global food and nutrition security.

**4. ZINC AND IRON: AN IMPERATIVE MICRONUTRIENT**

Zinc (Zn) is an essential micronutrient and one of the most abundant transition metals, playing crucial roles in both human and plant health. It acts as a co-factor for over 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids, and is vital for functions such as gene expression regulation and protein folding **(Wani *et al*., 2017)**. Zinc deficiency can lead to a range of health issues, including muscle weakness, skin problems, hair and memory loss, delayed puberty, nail dystrophy, diarrhoea, pneumonia in children, growth retardation, hypogonadism, erectile dysfunction, weakened immunity, alopecia, and an increased risk of cancer (**Nakandalage *et al*., 2016).** In plants, zinc is essential for growth hormone production, internode elongation, and numerous metabolic functions. It activates enzymes such as carbonic anhydrase, RNA polymerases, and alcohol dehydrogenase **(Palmer and Guerinot, 2009).** Zinc deficiency in plants can cause: Interveinal chlorosis, striping and mottling on leaves, pale gray-white leaves, premature leaf shedding and plant death in severe cases **(Kobayashi and Nishizawa, 2012).**

Iron deficiency is a common constraint on plant growth and yield. Although soils contain significant iron, its availability to plants can be limited by alkaline pH, which causes iron to precipitate into insoluble forms. Iron is crucial for chlorophyll and cytochrome production, playing vital roles in respiration and photosynthesis, and is a key component of enzymes like peroxidase, catalase, and nitrogenase. It also aids in nitrogen fixation alongside molybdenum. Iron deficiency in plants impairs chlorophyll production, leading to interveinal chlorosis in young leaves, which may progress to a whitish-yellow appearance and necrosis as the deficiency worsens **(Lucena and Hernandez, 2017**). In living organisms, iron is essential for catalytic functions of enzymes involved in oxygen transport, electron transfer, redox reactions, collagen biosynthesis, and vitamin D metabolism **(**[**Toxqui and Vaquero, 2015**](https://www.frontiersin.org/articles/10.3389/fpls.2021.647341/full#B112)**).** Iron deficiency anemia (IDA) affects all population segments, with children and pregnant women being particularly vulnerable ([**World Health Organization, 2008**](https://www.frontiersin.org/articles/10.3389/fpls.2021.647341/full#B123)).

**5. . CONCEPTION OF BIOFORTIFICATION**

Biofortification is a sustainable agricultural strategy aimed at combating zinc (Zn) and iron (Fe) deficiencies in food sources by enhancing the nutritional content of crops (**Figure 1**). By breeding crops with higher micronutrient levels and using agronomic practices such as foliar or soil applications, biofortification significantly boosts micronutrient intake and benefits human health. An innovative method within biofortification is **microbe-mediated fortification**, which utilizes plant-microbe interactions to improve soil nutrition and increase micronutrient uptake. This eco-friendly approach involves metal solubilization, mobilization, and translocation within the plant, resulting in healthier, more nutrient-rich crops **(Melnick *et al*., 2011; Sharma *et al*., 2013; Wang *et al*., 2014; Garg *et al*., 2018; Singh and Prasanna, 2020).** Additionally, biofortification not only enhances micronutrient content but also stimulates the synthesis of other beneficial compounds, further enriching the nutritional quality of crops. This comprehensive approach offers a promising solution to address global micronutrient deficiencies sustainably **(Huang *et al*., 2020; Praharaj *et al*., 2021).**

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**Figure 1:- Overview of microbe mediated biofortification of zinc and iron (PGPB – Plant growth promoting bacteria; ZIP – Zinc regulated transporter; IRT – Iron regulated transporter; YSL – Yellow stripe like transporter**

**5.1AGRONOMIC METHODS:**

Agronomic biofortification enhances crop micronutrient levels through soil or foliar fertilizer applications. While soil fertilization is common, it has limitations like low efficiency and uneven nutrient distribution. Foliar fertilization, spraying nutrients directly on leaves, ensures faster and more uniform absorption, with studies showing its superiority, such as a 1.7-fold increase in zinc (Zn) concentration in mungbean grains compared to soil application (**Haider et al., 2018a, 2018b**). Combining soil and foliar methods or multiple nutrients like Zn, iron (Fe), and selenium (Se) further improves outcomes (**Gomez-Coronado et al., 2016**). Organic zinc and iron complexes outperform inorganic fertilizers in yield and nutrient content (**Montoya et al., 2020**). Nanotechnology offers advances, with zinc oxide nanoparticles (ZnONPs) and nano iron boosting nutrient levels and crop growth while reducing fertilizer dependence (**Subbaiah et al., 2016**). However, overreliance on fertilizers can harm the environment, making balanced, sustainable practices crucial (**Prasad and Babu, 2017**).

**5.2PLANT BREEDING:**

Plant breeding for biofortification employs traditional and modern strategies to enhance nutritional traits. Traditional breeding involves crossing donor plants with desired traits and recipient plants with strong agronomic features, using populations like F2, backcrosses (BCs), and recombinant inbred lines (RILs) for genetic mapping and QTL identification (**Koç and Karayiğit, 2022; Kumar et al., 2023**). While effective, it is time-consuming and limited by genetic diversity, which can be addressed by using landraces or wild relatives with higher nutrient density (**Medina-Lozano and Díaz, 2021**). To overcome limitations like linkage drag and inefficiency, genomic selection and Marker-Assisted Selection (MAS) are used to target traits like elevated zinc in wheat or carotenoid alteration in sorghum (**Cruet-Burgos et al., 2020**). MAS facilitates rapid crop improvement by tracking genes for nutrient homeostasis and efficiency (**Chandra et al., 2020**). Programs like CIMMYT and CIP have developed biofortified crops, such as provitamin A-rich sweet potatoes and maize with high lysine and tryptophan levels (**Prasanna et al., 2020**). Advances in molecular markers have further identified QTLs linked to enhanced nutritional content in crops like beans, wheat, and cereals (**Garg et al., 2021**).

**5.3TRANSGENIC APPROACH:**

Transgenic techniques in biofortification transfer alleles responsible for enhanced nutritional value from different species to target crops. This approach overcomes the limited genetic alternatives in traditional breeding confined to sexually compatible species (**Brinch-Pedersen et al., 2007**). Transgenesis allows the expression of genes regardless of their origin (**Coca et al., 2006**), making it the only method to create crops producing nutrients not naturally synthesized by them. This expands the gene pool and enables the transfer of multiple genes and regulatory elements simultaneously. Successful examples include nutrient-enriched rice varieties (**Singh et al., 2017**). However, some crops, like cereals and legumes resist alteration (**Yadav et al., 2020**). Transgenic techniques require significant time and resources for gene identification and introduction, and their widespread adoption is limited by high costs and concerns about cross-pollination, contamination, safety, and environmental impact, which hinder acceptance and uptake.

**5.4 MICROBIAL AIDED BIOFORTIFICATION**

Microbe-mediated fortification is a quick and straightforward method to enhance nutrient content in food crops, enriching them with essential elements like metal ions (Zn, Fe, Se), vitamins (A, B, C), and fatty acids (**Kumari et al., 2023**). While plants have developed ways to acquire vitamins, they often struggle to prevent micronutrient deficiency in nutrient-poor soils. Soil microorganisms involving Bacillus, Pseudomonas, Azotobacter, Rhizobium, and Azospirillum are plant growth-promoting microorganisms (PGPM), play a crucial role in nutrient cycling and mineral uptake by converting complex soil minerals into bioavailable forms, aiding crop biofortification (**Gosal et al., 2010**). Beneficial fungi like arbuscular mycorrhizal (AM) fungi, Trichoderma, and Penicillium enhance nutrient uptake by accessing minerals beyond the reach of plant roots (**Mishra et al., 2023**). Zinc-solubilizing bacteria (ZSB) in the rhizosphere improve plant nutrient absorption by dissolving complex zinc compounds, facilitating eco-friendly biofortification (**Saravanan et al., 2007; Sharma et al., 2013; Shakeel et al., 2015; Upadhayay et al., 2022a**). Bacterial strains such as *Bacillus aryabhattai*, *Bacillus subtilis*, *Burkholderiacepacia*, and *Pantoearodasii* are effective bio-inoculants for zinc biofortification in wheat, maize, and rice. Zn- and P-solubilizing bacteria like *Rhizobium radiobacter* (LB2) have improved zinc nutrition in lettuce, even in saline soils (**Ramesh et al., 2014; Costerousse et al., 2017; Hussain et al., 2018; Mumtaz et al., 2020; Kushwaha et al., 2021; Upadhayay et al., 2022b**). PGPMs produce siderophores, which chelate metal ions like iron, improving uptake, especially in stressed soils. Siderophore-producing bacteria such as Bacillus, Pseudomonas, Streptomyces, Azotobacter, Gluconacetobacter, and Azospirillum positively impact plant health (**Delaporte-Quintana et al., 2020; Ghazy and El-Nahrawy, 2021**). Fungi like Cymbidium, Penicillium, Aspergillus, and Trichoderma also produce siderophores, enhancing iron uptake in the rhizosphere (**Mukherjee et al., 2018; Chowdappa et al., 2020**). In a study by **Mushtaq et al. (2023)**, *Acinetobacter calcoaceticus* and *Bacillus simplex* increased iron content in potato tubers by 68.24% compared to control plants, highlighting microbial-assisted biofortification's. **Kartik et al. (2023)** found that siderophore-producing bacteria significantly enhanced rice plant growth, with iron mobilization in grains ranging from 53.88% to 89.05%, demonstrating significant improvements over uninoculated plants.

 **Table 1: Benefits of Zn and Fe solubilizing bacteria in crop biofortification**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No.**  | **Bacterial Treatment** | **Crop** | **Benefit to crop** | **Amount of Zn/ Fe in edible part** | **References** |
|  | *Bacillus glycinifermentans*CRS-9, *Microbacteriumoxydans* CRS-17, *Paenarthrobacternicotinovorans*CRS-30, and *Bacillus tequilensis*CRS-38 | Wheat | Enhanced germination rates, vigor indices, plant biomass, grain yield, and zinc content in grains | Zn: 54.25 μg/g  | **Yadav et al., (2022)** |
|  | *Pseudomonas jesenni* MP1 and *P. palleroniana* N26 | Kidney bean | Improves kidney bean yield, nutrient profile, and soil health | Zn: 53.66 mg/kg50.16 mg/kg | **Khan et al., (2023)** |
|  | *Burkholderiacepacia* BMRR126 and *Pantoearodasii*BMAR64 | Rice | Resulted in the highest growth and productivity of the paddy crop | Zn: 33.25 mg/kg | **Upadhayay et al., (2022)** |
|  | *Arthrobactor* sp. | Soyabean | Increased grain yield and also enhanced Fe concentration in grains | Fe: 0.11 mg/g | **Sharma et al., (2019)** |
|  | *Lysinibacillus fusiformis*  | Wheat | Increased grain yield of wheat, enhanced Fe concentration of grains | Fe: 0.222 mg/g  | **Sharma et al., (2019)** |
|  | *Bacillus mojavensis* RHPR20 and *Bacillus cereus* MMRH22 | Sorghum | Significant increase in the plant height, leaves number, leaf area; root, shoot, and leaf weight; and the yield  | Fe: 87.5 ppm MMRH22 and 78.1 ppm RHPR20 | **Manasa et al., (2021)** |
|  | *Pseudomonas bijieensis* and *Priestia megaterium* | Finger millet | Improve the shoot and root lengths and the plant and root dry weights, zinc content and NPK content in grains controls | Zn: 2.73 mg | **Chaudhary et al., (2023)** |
|  | *B. aryabhattai* ZM31 and *B. subtilis* ZM6 | Maize | Increase nutrients acquisition and to promote growth and yield of maize | 52.0 mg/kg | **Mumtaz et al., (2020)** |
|  | *Pseudomonas monteilii* SRI-360 | Chickpea  | Improves yield traits including nodule number, nodule weight, shoot weight, root weight and grain yield  | Zn :3.9 mg/ 100 gFe: 6.1/100 g | **Gopalakrishnan et al., (2016)** |
|  | *E. ludwigii* SRI-229 | Pigeonpea | Improves yield traits including nodule number, nodule weight, shoot weight, root weight and grain yield | Zn :3.8 mg/100gFe: 3.9 mg /100 g | **Gopalakrishnan et al., (2016)** |
|  | *Bacillus subtilis*+Fe | Groundnut |  Increase growth, yield and nutrient contents  | Fe: 45.72% | **Sarwar et al., (2022)** |
|  | *Exiguobacteriumaurantiacum* MS-ZT10 | Wheat | Enhanced micronutrient content in wheat varieties | Zn: 18.2 ppm Fe: 24.67 ppm | **Shaikh and Saraf, (2017)** |
|  | *Alcaligenes sp., Bacillus sp., Pseudomonas sp. and Bacillus sp.* | Maize | Increased Zn contents in grain, cob-pith, stem and roots | Zn: 7.30 ppm | **Bashir et al., (2021)** |

**6.ENDOPHYTES AS PGPR AND BIOFERTILIZERS**

Plants host a diverse range of microbes, including epiphytes and endophytes. Endophytes, consisting of symbiotic bacteria and fungi, reside within plant tissues such as stems, roots, leaves, seeds, and fruits without harming the plant. They have been found in various plants and are categorized as transient or true endophytes. True endophytes are further divided into obligate (fully dependent on the plant) and facultative (partially dependent) types **(Choudhary et al., 2023**). Endophytes are of particular interest due to their ability to produce bioactive compounds with applications in agriculture, pharmaceuticals, and biotechnology. They enhance plant health by improving nutrient absorption, producing phytohormones, and combating pathogens through antibiosis and enzyme activation (**Choudhary et al., 2023**). Their internal presence in plant tissues makes them more effective in promoting plant growth and suppressing diseases than rhizospheric microorganisms (**Weyens et al., 2013**). Bacterial and fungal endophytes are linked to enhanced iron (Fe) and zinc (Zn) biofortification in wheat and rice grains (**Abaid-Ullah et al., 2015**). Endophytes enhance plant growth through mechanisms such as solubilizing immobilized nutrients like phosphorus and zinc, fixing nitrogen, producing phytohormones (auxins, cytokinins, gibberellins), sequestering iron via siderophores, oxidizing sulfur, producing ACC deaminase, and generating volatile growth stimulants like acetoin and 2,3-butanediol. Indirectly, they promote growth through antibiosis, siderophore production, induced systemic resistance (ISR), resource competition, hydrogen cyanide (HCN) production, and releasing cell wall-degrading enzymes. These growth-promoting endophytes increase nutrient availability, indirectly enhancing crop growth and yield (**Patle et al., 2018**).

**7.MICROBE INTERVENED MECHANISMS IMPLICATED IN PLANT GROWTH PROMOTION AND FOR UPTAKE AND TRANSLOCATION OF MICRONUTRIENTS IN PLANTS**

PGP microbes enhance plant growth through direct and indirect mechanisms (**Figure 2**). Directly, they assist in the acquisition of essential nutrients like nitrogen, phosphorus, potassium, zinc, and iron and regulate plant hormones such as auxin, gibberellin, cytokinin, and ethylene. Indirectly, they protect plants from pathogens like bacteria, fungi, and nematodes by producing antibiotics, releasing cell wall-degrading enzymes, modulating ethylene levels, inducing systemic resistance, reducing pathogen access to iron, and synthesizing pathogen-inhibiting volatile compounds. Organic acid synthesis is key to phosphate and zinc solubilization. Additionally, the metabolic activities of bacteria and environmental conditions impact zinc solubilization. While autotrophic bacteria are known for metal solubilization, recent research highlights heterotrophic bacteria's which can enhance plant productivity by increasing zinc and iron in edible plant parts (**Upadhayay et al., 2018; Ramesh et al., 2014; Costerousse et al., 2017; Upadhayay et al., 2022b**).

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**Figure 2: - Representation of plant growth promotion by bacteria**

**HCN - Hydrogen cyanide; EPS – Exopolysaccharide; ISR – Induced systemic resistance**

**7.1PRODUCTION OF SIDEROPHORE AND OTHER CHELATING AGENTS**

Siderophores are small molecules with three iron-binding groups, typically containing two oxygen atoms (or nitrogen) that form a six-coordinate complex with trivalent ferric iron. Fungi often produce hydroxamate-type siderophores, while bacterial siderophores typically have catecholate groups with stronger iron-binding affinity. Bacterial siderophores can also bind other metal ions but with lower affinity (**Olanrewaju et al., 2017**). Siderophores enhance iron solubility by forming siderophore-Fe complexes (**Desai & Archana, 2011**) and restrict pathogen growth by outcompeting pathogen siderophores for iron (**Singh et al., 2022**). **Sharma et al. (2003)** found that Pseudomonas strain GRP3 improved iron nutrition and chlorophyll content in ***Vigna radiata***. **Karthik et al. (2017)** reported that 35% of leaf endophytic bacteria, including **Pantoea, Pseudomonas, Rhodococcus, Serratia,** and **Klebsiella**, produced siderophores that boosted tomato growth. Furthermore zinc bioavailability is enhanced by Zn chelating agents, either synthetic or produced by plant roots and rhizosphere microbes (**Obrador et al., 2003**). These agents chelate Zn, increasing its uptake by reducing its soil reactivity (**Tarkalson et al., 1998**). Microbes like ***Microbacteriumsaperdae*, *Pseudomonas monteilii*,** and ***Enterobacter sp.*** improve Zn uptake through chelation. ***Azospirillumlipoferum*, Pseudomonas,** and **Agrobacterium** act as biofertilizers, mobilizing Zn with chelating agents like EDTA (**Tariq et al., 2007**).

 **7.2ZINC (ZN) SOLUBILIZATION**

Zinc is an essential micronutrient for plant growth. To increase zinc availability in the soil, Zn-solubilizing rhizobacteria are a valuable alternative. Bacteria such as Pseudomonas protegens (**Yasmin et al., 2021**), Bacillus megaterium (**Bhatt and Maheshwari, 2020**), Enterobacter cloacae (**Krithika and Balachandar, 2016**), Bacillus altitudinis (**Kushwaha et al., 2021**), and Bacillus spp. (**Ramesh et al., 2014**) have been identified as plant growth-promoting bacteria (PGPB) that produce plant hormones and growth factors, facilitating zinc availability to plants. The mechanisms of Zn-solubilizing bacteria include acidification via organic acids, metal-chelating siderophore synthesis, and oxidation-reduction activation (**Upadhayay et al., 2022**). Specifically, Burkholderiacepacia and Pantoearodasii exhibit halo zone formation on zinc-supplemented media, indicating acidic conditions favourable for zinc solubilization. In rice cultivation, these bacteria increased zinc content in grains, reaching 25.07 mg/kg and 33.25 mg/kg, enhancing zinc availability and uptake (**Upadhayay et al., 2022b**). **Srithaworn et al. (2023)** evaluated Priestia megaterium and Priestiaaryabhattai for Zn solubilization, forming zones on agar medium. They solubilized Zn at rates of 1.32 to 2.84 for zinc oxide and 1.93 to 2.27 for zinc carbonate. Inoculation with these isolates increased soybean grain yield by 48.97% and 35.29%.

**7.3PRODUCTION OF HORMONAL COMPOUNDS**

Bacteria produce a variety of phytohormones, including auxins, cytokinins, and gibberellic acids, which are crucial for plant growth and regulation. Among these, indole-3-acetic acid (IAA) is the most active auxin, playing a vital role in plant physiological processes such as geotropism, phototropism, cell elongation, apical dominance, and root system development. IAA enhances root biomass, nutrient uptake, and nodule development, as well as regulates stomata size and density (**Ahemad and Kibret, 2014**). Most IAA is synthesized from the amino acid tryptophan, present in plant root exudates, through three biosynthetic pathways: the indole pyruvic acid (IPyA) pathway, the indole acetamide (IAM) pathway, and the indole acetaldoxime (IAOx)/indole acetonitrile (IAN) pathway (**Duca et al., 2014**). Research by **Kuklinsky-Sobral et al. (2004)** revealed that endophytic bacteria from soybean cultivars can synthesize IAA***. Burkholderiavietnamiensis***, a diazotrophic bacterium from wild cottonwood (Populustrichocarpa), was found to synthesize IAA, leading to increased plant dry weight and nitrogen content (**Xin et al., 2009**). **Khianngam et al. (2023)** identified two endophytes, ***Enterobacter hormaechei*** and ***Bacillus aryabhattai***, capable of producing significant IAA levels, eliminating the need for synthetic IAA and promoting sustainable agriculture. Gibberellins, another group of phytohormones, are tetracyclic diterpenoid carboxylic acids that regulate seed germination, seedling emergence, leaf and stem growth, flower development, and fruit size. They also promote root and stem growth and delay senescence (**Rana et al., 2020**). Several bacterial genera, such as **Azospirillum, *Achromobacterxylosoxidans*, *Acetobacter diazotrophicus*, Bacillus, *Herbaspirillumseropedicae***, and **Rhizobium**, produce gibberellins (**Gutiérrez-Mañero et al., 2001; Deka et al., 2015**). Of the 136 known gibberellin structures, only four (GA1, GA3, GA4, and GA20) are found in bacteria, with GA1 and GA4 showing the highest activity (**Nelson and Steber, 2016**). This remarkable ability of bacteria to produce phytohormones highlights their potential to promote plant growth naturally, offering sustainable solutions for agriculture.

**7.4 ORGANIC ACIDS PRODUCTION IN ROOT EXUDATES**

Plant root exudates enhance nutrient solubility in the soil through various biochemical processes **(Zhang et al., 2010)**. Microorganisms influence root exudation patterns, affecting rhizosphere interactions (**Da Silva et al., 2014**). Organic acids, a key component of root exudates, play a significant role in metal solubilization (**Luo et al., 2014**). Soil pH strongly impacts micronutrient availability, with a one-unit pH increase drastically reducing zinc and iron solubility (**Havlin et al., 2007**). Microbial activity in the rhizosphere, particularly from organic acid-producing microbes, enhances nutrient availability, particularly phosphorus, potassium, and zinc (**Sirohi et al., 2015**). Inoculation with plant growth-promoting rhizobacteria alters root exudate patterns, boosting metal solubilization (**Chen et al., 2014**). Bacteria contribute to nutrient availability by acidifying the rhizosphere, similar to the role of organic acids. Phosphorus-solubilizing fungi and bacteria further enhance phosphorus availability by lowering soil pH (**Asea et al., 1988**). Studies show that bacterial inoculation, such as with Arthrobacter strains, increases zinc and iron levels in plants, likely due to changes in organic acid profiles (**Singh et al., 2017**). Similarly, strains like Bacillus sp., Pseudomonas taiwenensis, and B. aryabhattai promote zinc solubilization, improving plant growth (**Vidyashree et al., 2018**). Organic acid release by Enterobacter has also been shown to enhance phosphorus uptake in cucumber plants (**Zuluaga et al., 2023**).

**7.5 CHANGES IN ROOT MORPHOLOGY AND ANATOMY**

Plant growth-promoting rhizobacteria (PGPR) enhance root system functioning, improving crop resource use, stress tolerance, and soil structure (**Grover et al., 2021**). PGPR-mediated changes in root traits, like increased root hairs, surface area, and metal-mobilizing exudates, boost nutrient uptake (**Genc et al., 2006**). Zinc-efficient plants develop finer roots, enhancing Zn uptake (**Chen et al., 2009**). Inoculating wheat with Zn-solubilizing bacteria (Bacillus subtilis DS-178) doubles Zn grain accumulation, rivalling ZnSO₄ application (**Singh et al., 2017a**). Siderophore-producing bacteria (Arthrobacter sulfonivorans DS-68) improve root traits and Fe uptake (**Singh, 2016**). Microbial inoculants enhance root anatomy, including xylem and cortex dimensions, boosting nutrient absorption (**Singh et al., 2017b**). **De *et al*. (2023)** reported that *Trifolium repens* and *Vicia sativa*, when subjected to stress and concurrently inoculated with rhizobial bacteria, exhibited enhanced plant growth and increased root complexity compared to the control group.

**7.6 OVER EXPRESSION OF METAL TRANSPORTER:**

Micronutrient uptake and translocation are distinct processes, with some crop genotypes showing efficient uptake but poor movement of micronutrients to grains (**Singh et al., 2018**). Effective management of nutrient translocation is crucial for enhancing micronutrient content in edible plant parts. Metal transporters like the ZIP family, CDF family, and P-type ATPases play key roles in zinc and iron translocation, including xylem loading (**Colangelo and Guerinot, 2006**). Overexpression of ZIP proteins in crops like wild emmer wheat increased zinc accumulation (**Durmaz et al., 2011**). In rice, Zn-solubilizing Enterobacter cloacae ZSB14 enhanced the expression of OsZIP1 and OsZIP5 while reducing OsZIP4 expression (**Krithika and Balachandar, 2016**). Rhizophagusirregularis upregulated HvZIP13 in barley under Zn-deficient conditions, increasing grain Zn concentrations (**Watts-Williams and Cavagnaro, 2018**). Similarly, Arthrobacter sp. improved TaZIP3 and TaZIP7 expression in wheat roots and shoots, enhancing Zn translocation (**Singh et al., 2017b**).

**7.7 PHOSPHATE SOLUBILIZATION:**

Phosphorus (P) is essential for plant health, but much of it in soil is insoluble and inaccessible to plants. Phosphate-solubilizing bacteria, such as *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Rhizobium*, convert these forms into bioavailable P through acidification, chelation, or enzymatic processes (**Richardson et al., 2009**). Endophytic bacteria, including *Achromobacterxiloxidans* and *Bacillus pumilus*, effectively solubilize phosphates, as shown in sunflower and soybean studies (**Forchetti et al., 2007**). In maize, the use of phosphate-solubilizing bacteria reduced phosphorus fertilizer requirements by 50% without lowering yields (**Yazdani and Bahmanyar, 2009**). **Varga et al. (2020)** visualized phosphate solubilization in poplar roots inoculated with endophytes using synchrotron X-ray fluorescence, revealing increased root biomass, novel proteins, and metabolic pathways linked to improved P uptake.

**7.8 BIOLOGICAL NITROGEN FIXATION:**

Plants acquire essential nitrogen (N) from soil as nitrite (NO₂⁻), nitrate (NO₃⁻), or ammonia (NH₄⁺), but these forms are often scarce, and chemical fertilizers are prone to losses through leaching. Leguminous plants like soybean, pea, and peanut establish symbiotic relationships with nitrogen-fixing bacteria (e.g., Rhizobium and Bradyrhizobium), forming root nodules that convert atmospheric nitrogen (N₂) into ammonia, providing the plant with accessible nitrogen (**Murray, 2011**). Non-symbiotic, free-living N₂-fixing bacteria (e.g., Azospirillum*,* Azotobacter*,* Bacillus polymyxa*,* Burkholderia*,* Herbaspirillum) act as plant growth-promoting rhizobacteria (PGPR) and enhance the growth and yield of crops like wheat, maize, and sugarcane (**Malik et al., 1997**). For instance, Azoarcus*sp.* support Kallar grass in N-deficient soils (**Reinhold-Hurek et al., 1993**), Klebsiella sp. improves maize and wheat yields (**Riggs et al., 2001**), and nitrogen-fixing endophytes mitigate N-deficiencies in sweet potato (**Reiter et al., 2003**). Similarly, Burkholderiaendophytes in grasses supply nitrogen via nitrogenase activity (**Dalton et al., 2004**). Recent studies highlight biofertilizer potential. **Zhang et al. (2022)** identified nitrogen-fixing endophytes in cassava with high nitrogenase activity, while **Devi et al. (2022)** isolated strains like Erwinia persicina and Halomonasaquamarina that enhanced chili plant growth through nitrogen fixation and mineral solubilization. These findings emphasize the promise of nitrogen-fixing microbes in sustainable agriculture.

**7.9 POTASSIUM SOLUBILIZATION:**

Potassium-solubilizing microorganisms (KSMs) serve as biofertilizers, reducing pesticide use and enhancing crop yields. **Meena et al. (2016)** reported that KSMs increased potassium availability in soil from 84.8% to 127.9%. While less studied, fungi like Penicillium pinophilum and yeasts such as Torulasporaglobosa also act as effective K solubilizers, boosting pomegranate yields by 116.9% (**Maity et al., 2019**). KSMs offer additional benefits, including phytohormone production (e.g., IAA, cytokinins), N fixation, P solubilization, ACC production, and pathogen control (**Soumare et al., 2021; Gopalakrishnan et al., 2020**).

**7.10 EXO POLYSACCHARIDES (EPS) PRODUCTION OR BIOFILM FORMATION:**

Exopolysaccharide (EPS)-producing bacteria play vital roles in plant-microbe interactions by enhancing biofilm formation, stress resilience, nutrient cycling, and enzyme activity while improving soil aggregation and plant defense responses (**Deka et al., 2019***).* **Dar et al. *(2021)*** demonstrated that EPS-producing Rhizobium phaseoli and Pseudomonas bathysetes significantly boosted maize growth, yield, and soil properties. Similarly, **Prasad et al. (2022)** found that biofilm-forming bacteria, including Pseudomonas azotoformans and Burkholderiaseminalis, enhanced wheat germination, growth, and yield through biopriming. These improvements were linked to key PGP traits like phytohormone production, phosphate solubilization, and EPS synthesis.

**7.11 BIOCONTROL**

Microbes play a crucial role in enhancing plant protection against diseases by producing secondary metabolites like flavonoids, peptides, alkaloids, and terpenoids with antifungal and antibacterial properties (**Lugtenberg et al., 2016**). Enzymes such as chitinases, cellulases, and glucanases aid in degrading plant and fungal cell walls, reducing pathogen viability (**Fadiji and Babalola, 2020**). Additionally, many microbes produce ACC deaminase, mitigating the harmful effects of ethylene buildup during pathogen attacks (**Arshad et al., 2007**). Induced systemic resistance (ISR) facilitated by microbes enhances plant defense and nutrient acquisition, including iron mobilization through MYB72-regulated phenolic secretion (**Romera et al., 2019**). Beneficial bacteria such as Bacillus subtilis, Pseudomonas protegens, and Burkholderiapyrrocinia demonstrate antagonistic activity against fungal pathogens like Botrytis cinerea and Fusarium species by producing antifungal compounds and volatile organic substances (**Baard et al., 2023**). Microbial applications in crops like potato, soybean, and tomato have significantly reduced disease severity while promoting plant growth, highlighting their potential as effective biocontrol agents (**Bahmani et al., 2021; Win et al., 2022**).

**8. CONCLUSION**

Microbe-mediated biofortification presents a sustainable and eco-friendly strategy to combat micronutrient deficiencies, particularly iron and zinc shortages in plants and humans. By leveraging beneficial microorganisms, this approach enhances soil fertility, nutrient bioavailability, and crop nutritional quality. Unlike conventional methods, such as chemical fertilization and supplementation, microbial biofortification minimizes environmental impact while offering long-term benefits. Studies have demonstrated its efficacy in improving Fe and Zn uptake in staple crops, thereby contributing to global food security. However, challenges such as microbial strain stability, field-level efficacy, and scalability must be addressed for broader implementation. Integrating microbial biofortification with conventional breeding and agronomic practices can further enhance its effectiveness. Continued research and policy support are crucial to optimizing microbial formulations for diverse agroecosystems. Additionally, farmer awareness and adoption play a key role in realizing the full potential of this strategy. Overall, microbe-mediated biofortification holds immense promise for addressing malnutrition and improving agricultural productivity. Future advancements in microbial technology can further refine and expand this approach for global nutritional security.

**DECLARATION OF INTERESTS**

* The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.
* The authors declare the following financial interest or relationship that may be considered potential competing interests.

**DATA AVAILABILITY STATEMENT**

No datasets were generated or analysed during the current study.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist

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