

Role of Soil Rhizobacteria in Utilization of an Indispensable Micronutrient Zinc for Plant Growth Promotion

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ABSTRACT: The crop yield production depends on different nutrients (macro and micronutrients), but because of the several anthropogenic and environmental factors, the plants face some micronutrient deficiencies. Among them, worldwide the zinc (Zn) deficiency are common (mainly in the arid and semi-arid regions characterized by calcareous soils). These elements have a high concentration in the soils of these areas, however, their absorbable concentration for the plant is low (because of several factors, such as high soil pH, lack of organic matter, high bicarbonate in soil solution, compaction and lack of soil aeration and high consumption of phosphorus fertilizers) and subordinate values of micronutrients would reduce crop efficiency and yield. To overcome the Zn deficiency, chemical fertilizers are used. Extreme usage of the chemical fertilizers decreases soil fertility and makes the crops more susceptible to disease. There are reports of potential for insoluble zinc dissolution in the soil by rhizospheric microbes. These microbes can increase the supplying of nutrients, including soil insoluble zinc, to the plant through mechanisms such as the proton secretions, production of siderophores and organic and mineral acids. In order to achieve sustainable agriculture and the use of environmentally friendly solutions, the microbial potentials and their metabolites, as environmentally friendly agents and their replacement by chemical compounds is of particular interest to overcome the problem of supplying zinc needed by plants in calcareous soils. This study critically evaluates the soil microbe's effect on the insoluble zinc dissolution in the soil, which can increase the bioavailability of these elements for plants and prevent soil fertility reduction due to overuse of chemical fertilizers.

Keywords: Plant rhizosphere, zinc nutrient, solubilization, plant growth promotion.

Generally, because of inadequate dietary intake, the incidence of micronutrient deficiencies remains high (Gregory *et al.*, 2017). More than 2 billion people in the world are exposed to Zn micronutrient deficiencies, especially in developing countries (Joy *et al.*, 2014; Nutrient stress can lead to plants suffering when the soil nutrients availability and/or the amount of nutrients taken up in a particular growth stage is below that required to sustain metabolic processes (Rengel, 2015). This can result from an inherently low soil nutrient status, low soil nutrient mobility, poor solubility of the nutrient's given chemical type, or interactions between soil and microbes (Marschner *et al.*, 2011). The abundance of nutrients inside the rhizosphere is regulated by combined soil properties effects, plant characteristics, and the relationships between the plant and the microorganisms and the soil. Increased availability of short-chain organic acid (OA), amino acids, and other low molecular weight organic compounds can result from solubilization and mobilization by anions. Rhizospheric soil acidification improve mobilization of micronutrients (Zn-100 times the increase in solubility for each pH-decrease unit) (Rengel, 2015). In plants, Zn requirement is 30-mg kg⁻¹, below which its deficiency will lead. Zn conducts different biochemical and metabolic roles in a plant. It is a constituent of ribulose 1, 5-biphosphate carboxylase (RuBPC), carbonic anhydrase which are essential in photosynthesis. Zn deficiency results in a decrease in net photosynthesis by 50-70 per cent. Zn also plays a role in the synthesis of RNA, DNA and the regulations. It defends against attack by ribonuclease ribosomal RNA. Zn has ribonuclease activity, and it also inhibits lipid and protein peroxidation attributable to ROS. In plants genes responsible for tolerance to environmental stress are based on Zn. Stunted development, chlorosis, smaller leaves, short internodes (in cereals) and leaf rosetting are obvious signs of Zn deficiency. Younger leaves are primarily affected, and may visualize interveinal chlorosis symptoms. Zn deficiency can be related to various factors affecting total soil Zn content as shown in Figure 1.

II. ZINC AND SOIL

Forms of Zinc in Soils

Crops need the micronutrient zinc for healthy growth and normal reproduction. In addition Zn, other elements such as iron, copper, manganese, molybdenum, boron, nickel, and chlorine are needed in small amounts for the plant but are absolutely necessary and vital for plant growth. These elements are required in relatively small tissue concentrations (5-100 mg kg⁻¹). In addition to plants, many of these elements are essential for humans and animals, such as iron, zinc, copper, and manganese. In addition the eight trace

elements, nine main components (present at far higher concentrations, > 0.1 per cent) are also important for plants: carbon, oxygen, hydrogen, phosphorus, nitrogen, potassium, magnesium, calcium, and sulphur (Alloway, 2008; Vodyanitskii, 2010). Zinc exists in the walls, obviously. The amount of zinc present in the soil is dependent on the soil's parent materials. For general, sandy and heavily leached acid soils have low zinc available to plants. Mineral soils with low organic soil also show a deficiency in zinc. Soils from igneous rocks are richer in zinc, by comparison. Plants take in zinc as the ionic divalent (Zn^{2+}) and chelated-zinc form. In the soils the total zinc quantity is distributed over 5 pools (Alloway, 2008).

That include:

- i) Water-soluble pool: soil solution present,
- ii) Exchangeable pool: Ions bound by electric charge to soil particles,
- iii) Organically bound pool: Ions adsorbed with organic ligands, chelated or complexes with,
- iv) Zinc pool sorbed onto clay minerals and insoluble metallic oxides without exchange,
- v) This is only the zinc that is accessible in soluble fractions and from which the plants have ions readily desorbed and which can also be released into water percolating through the soil profile.

Zinc has the following types in soils:

- i) Zn^{2+} and $ZnOH^+$ ions free and zinc in solution organically complex,
- ii) Adsorbed and exchangeable zinc on colloidal soil surfaces, which contain clay, humic compounds, iron and aluminium oxides hydrated,
- iii) Secondary minerals and insoluble compounds during the soil stable period (Alloway, 2008).

The content of total zinc in the soils and its controllers factors

The cumulative zinc contents of a soil depend to a large degree on the geochemical composition of the soil's weathering rock parent material. For certain situations, though, the influence of the parent material may be blurred by environmental contamination and the use of zinc-rich agricultural products. The Earth Crust's total zinc content is 78 mg Zn kg⁻¹ and the maximum concentration of specific rock forms that make up the Earth's crust is zinc.

- Livestock Manures
- Fertilizers
- Industrial Waste Products
- Sewage Sludge
- Agrochemicals
- Atmospheric Deposition

The availability of zinc in soil for plants and the affecting factors

Plants have zinc available which is present or adsorbed in a labile state in the soil solution. Zinc is the field element that determines the quantity of zinc in the solution of the field and its sorption-desorption from / into the soil solution. The following influences include: overall zinc content, the pH, the quality of organic matter, clay content, calcium carbonate content, redox, rhizosphere microbial activity, the state of soil humidity, other trace materials, macro-nutrient amounts, in particular, phosphorus and atmosphere. In a specific sense for agriculture, some of these considerations are summed up: sandy soil and acid-laden leaking soils with poor overall and plant-available zinc are particularly susceptible to zinc deficiency. According to the rise in adsorption potential of zinc, hydrolysis process of zinc, probable chemical absorption by calcium carbonate and iron oxide, the supply of zinc decreases with increasing soil pH. Soils that are alkaline, calcareous, and highly modified tend to be less susceptible to zinc than soils that are neutral or mildly acidic. When organic matter such as manure is quickly broken down into soils, zinc may become more readily available due to the development of mobile and likely to absorb soluble organic zinc complexes in plant roots. Due either to the poor abundance of such organic materials and to the development of solid-state organic compounds, zinc concentrations may be small in the high-organic matter (peat and mud soils) soils. The supply of zinc or the occurrence of zinc deficiency associated with the fertilization of phosphorus may be attributed to physiological reasons for plants at elevated phosphorus levels. Some phosphatic fertilizers, such as superphosphate, are strongly zinc-like in impurities and can have an acidifying effect on soils. The occurrence of zinc deficiency has been shown to be growing frequently when substituted by 'fast study' sources of phosphatic fertilisers such as monoammonium phosphate (MAP) and diammonium phosphate (DAP). Lower soil copper concentrations compared to zinc will decrease the supply of zinc in a plant (including vice versa) as the plant root is consumed at the same location. It will happen when a copper fertilizer has been added. The reduced pH levels, elevated concentrations of bicarbonate ions, at times high magnesium concentrations, and the production of Zinc sulphide under highly reducing conditions result in porous soils such as paddy rice soils. Reduction in the frequently wet soil also

leads to the dissolution of their hydro oxides at increased divalent ferrous (Fe²⁺) and manganese (Mn²⁺) levels, which could compete with zinc ions to take root levels. The cumulative beneficial effect on crop plant nutrition can be achieved by providing both a nitrogen, sometimes the principal yield limiting nutrient, and also an increase in zinc abundance by acidifying the soil that results in zinc desorption, and by improvement in fundamental growth (and thus an increase in volume of soil), (such as ammonium and ammonia sulphate).

Where topsoil has been eroded, mostly as a result of irrigation levelling fields, crops grown on subsoil, particularly in calcareous soils, may be highly vulnerable to zinc deficiency. Most organic matter is found in the top soil and macronutrients and micronutrients are not usable until extracted. N, P and K fertilizers typically satisfy the criteria for macronutrients but attention must also be given to the zinc status of these 'broken' soils.

III. ZINC IN THE PLANTS

Physiological Functions of Zinc

In plant growth and production, Zinc is essential micronutrient (Zn). In all six groups of enzymes (oxidoreductase, transferase, hydrolases, lyases, isomerases and ligases), it is the only metal that is present. As a member of the structural or catalytic units, Zn is responsible for the functions of the various proteins (endymes), conformational stabilisation and folding. Including hormone control (e.g. tryptophane synthesis, an IAA precursor), signal transduction through mitogenic protein kinases, and maintenance of CO₂ concentrations in mesophyll, ZN has been involved in a number of plant physiological processes (e.g. tryptophan synthesis, IAA precursor) and in photoinhibition.

Mechanisms of Zinc Uptake by Plants

Zinc may be taken by plants as a divalent cation, but only a very small fraction is found as a soluble element in the soil. The majority of zinc is composed of insoluble mineral complexes (Alloway, 2008). Zinc deficiency occurs due to the absence of available surface zinc, one of the most common deficiencies in zinc. Different methods have been used for alleviating zinc deficiency for a long time. The use of zinc solubilizing rhizobacteria is a better alternative to all these approaches.

Zinc Deficiency in Plants

Zn's lack of food grain is a major cause of malnutrition which leads to inhibited human growth and unsuitable sexual development. There are several solutions used to enhance the availability of Zn in edible plant components, mainly through the use of various fertilizers and the development of Zn-efficient varieties through conventional and molecular breeding. The scientist's focus was on improving crop productivity and food quality, amongst nutrient management strategies, the promotion of plant growth by rhizobacteria or PGPR. In several studies PGPR has been demonstrated as a better replacement for hazardous chemicals to imitate Zn deficiency and increase field crop yields. They solubilize Zn by releasing the organic acids to soil and make it easier for the plant to use and use complex Zn compounds (Shakeel *et al.*, 2015). The main mechanism used for Zn solubilization by PGPR thus constitutes the production of organic acids.

Different approaches to solving the problem of zinc deficiency

Currently, to solve the problem of zinc micronutrient deficiency, the common strategy of farmers is to use chemical fertilizers containing zinc. Various approaches are being implemented to improve zinc concentrations in plants. One of these approaches is to improve the concentration of zinc in plants to eliminate zinc deficiency in humans. In addition to the use of zinc chemical fertilizers, the modification of plant genetics and the use of zinc solubilizing microbes to increase the bioavailability of zinc is also research in this field. Some factors increase the availability of zinc to the plant, such as organic acids (such as citrate), amino acids (such as histidine and methionine), and chelators (eg, EDTA), while some factors reduce the uptake of zinc such as minerals copper, Iron and calcium and some fiber. Various organic acids increase zinc plant uptake, such as malic acid, citric acid, ascorbic acid, and lactic acid. The reaction of the insoluble combination of phytate-zinc and EDTA can be used to form the zinc-EDTA complex. It has been reported that some rhizospheric ZSB, with other functions such as increasing the availability of phosphorus, potassium, iron as well as the production of phytohormones such as auxin, cytokinin and gibberellin, as well as production of metabolites such as siderophore and antibiotics and further inhibition of plant pathogens finally, they strengthen the growth of the plant. There are many reports of increased concentrations of plants inoculated with ZSB. Comparison of different approaches to supplying zinc for plants indicates that the use of ZSB to supplying zinc to plants due to its multiple positive effects as well as ecofriendly nature and low cost of production, can be a practical strategy to reduce the use of chemical products.

Plant Growth-Promoting Rhizobacteria and Enhancement of Zn Bioavailability

Plant growth-promoting rhizobacteria (PGPR) in the rhizosphere by utilizing direct and indirect mechanisms lead to increased plant growth (Sharma *et al.*, 2003). Direct mechanisms include providing nutrients to the plant by adopting strategies such as: biological nitrogen fixation, potassium release, phosphate solubilization, ACC deaminase activity, production of various phytohormones, siderophore, ligands, OAs, etc. (Fasim *et al.* 2002; Zhu and Yang, 2015; Shakeel *et al.* 2015). Indirect mechanisms include the inhibition of plant pathogens through the production of siderophore, hydrogen cyanide, antibiotics, enzymes, etc. (Glick, 1995). The rhizospheric Zn solubilizing bacteria may employ one or more of the mechanisms mentioned above. Decreasing the pH to increase the solubility of Zn is one of the most important mechanisms of these bacteria, which is made possible by the secretion of protons, production of organic and inorganic acids and various chelators (Goteti *et al.*, 2013; Vaid *et al.*, 2014; Idayu *et al.*, 2017) (Figure 1). For each unit decrease in pH, the solubility of Zn increase 1000 fold and 100 fold, respectively (Lindsay, 1979). Other mechanisms used by PGP bacteria to increase the solubility of Zn is siderophore production (Figure 1). Siderophores are organic low molecular weight and secondary metabolites that are produced by the presence of Fe³⁺ and to some extent by zinc, copper and manganese deficiency (Hussein and Joo, 2017). The synthesis of siderophore is regulated by the concentration of Fe in the medium and their production is restricted to a high concentration of iron. The high charge-to-radius ratio of Fe, as well as the presence of side chains and functional groups in the siderophore molecule, make this ligand highly preferred for the formation of the complex with Fe³⁺ (Sharma *et al.* 2003). Siderophores not only play a role in the supply of certain plant nutrients such as Fe and Zn, they can also play a role in controlling plant pathogens by removing the required iron for the pathogen, lead to weakening and death it through exposing the pathogens to iron deficiency (Lambrese *et al.*, 2018). In one experiment, it was found that *Bacillus* spp. dissolved insoluble zinc by proton secretion, OAs, phytohormones and chelating ligands (Wakatsuki, 1995). Production of OAs has been reported to be the major mechanism of Zn solubilization (Table 1). Among OAs producing gluconic acid (GA) and 2-ketoGA by PGPR leads to solubilization of zinc (Fasim *et al.*, 2002).

IV. MECHANISM OF PLANT GROWTH PROMOTING BY ZSB

The soil zinc in insoluble form can be convert to a soluble form via zinc solubilizing bacteria (ZSB) which makes it readily bio-available for plants growth, and development, while maintaining health and fertility of soil to sustainable agriculture. The solubility of Zn in the soil depends primarily on soil pH and humidity. Zinc is frequently deficient in arid and semi-arid areas. Carbon source, pH, and buffering capacity are primarily dictated by the quality and volume of the different organic acids formed by the various microorganisms on the soil (Mattey 1992). Metal salt solubilization is an important characteristic of PGPR as the mobilized compound is available to plants. Bacterial comparative and functional genomics research has exposed new approaches to molecular and biochemical underlying mechanisms. Several experiments have been performed to study Zn-solubilizing PGPR pathways. PGPR has various solubilization pathways, including reactions, chelation, and releases organic acids to solubilize nutrients in the soil (Chung *et al.* 2005). Siderophore formation (Burd *et al.* 2000) and gluconate production or acid derivatives, *i.e.* 2-keto-gluconic acid (Fasim *et al.* 2002). Among the mechanism of mobilization for the use of zinc, acidification is the most favored zinc solubilization process. However, as indicated in a long list of acid potential, any acid could solubilize Zn, including glucose, lactic, succinic, malic, propionic, 2- isobutyric, malonic, glyoxalic, fumaric, malic, ketogluconic, acetic, isovaleric, oxalic, itaconic, citric, tartaric, α - ketobutyric, glycolic, aspartic and gluconic acid; the acids that were most significantly increased during the growth of *Bacillus* sp. ZM20 and the *B. cereus* strain in the presence of ZnO were lactic and acetic, with significantly lower concentrations of other acids detected. Fasim *et al.*, 2002; Saravanan *et al.*, 2007) or any additional acid metabolites (tartaric and malic acids) have been found previously recorded in the process of solubilization of Zn (fluconic and 5 ketogluconic acids). The development of lactic acids is typically related to lactic acid non-aerobic bacteria, although there is no specific lactic formation of acid in the spore microorganisms aerobic or anaerobic (Fritze and Claus, 1995).

V. ZINC SOLUBILIZATION ASSAY AT IN-VITRO CONDITIONS

An adequate amount of rhizospheric soil in polyethylene bags are immediately transferred to the laboratory and air-dried (for two hours). After removing the soil, the roots of the plant are immersed in sterile water (in the antiseptic condition). This suspension is used to isolate bacterial strains in different media such as TSM, DF, Bunt and Rovira, etc. (Table 2) via serial dilution technique (Dinesh *et al.*, 2015). Since the purpose of the experiment is to isolate Zn solubilizing bacteria, the culture medium used to screen for these rhizosphere bacteria should be including insoluble zinc salts (Idayu *et al.*, 2017). After the emergence of bacterial colonies on the plate purified them and keep for further experimentation and store at low temperatures.

It has been reported that the range of the solubilization halo size by 8.67 - 13.33 mm in zinc oxide and by 1.00 - 5.33 mm in zinc phosphate medium (Kumar *et al.*, 2017). Similarly, it was reported that the solubilization halo ranging by 5 - 7.6 mm for the bacteria like *Pseudomonas*, *Azospirillum*, *Gluconacetobacter diazotrophicus*, *B. aryabhatai* and *Thiobacillus ferrooxidans* (Saravanan *et al.*, 2007; Saharan and Nehra, 2011; Gontia-Mishra *et al.*, 2017; Pourbabae *et al.*).

Quantitative Assessment of Zn Solubilization

To determine a quantitative amount of zinc dissolution, the media mentioned in the table 2 can be used that are containing insoluble Zn resources. 100 µL of bacteria-overnight cultures broth inoculate in Erlenmeyer flasks containing 50 ml medium with 0.1% insoluble zinc resources. The control sample will also contain a culture medium without bacteria inoculation. The samples are stored at 28°C for 14 days at a speed of 120°C. The samples are incubating for 14 days at 28°C with a rotational speed of 120 rpm. After end time, the samples are centrifuging at 6000 rpm, and the supernatant prepares for the measurement of dissolved zinc by atomic absorbance spectrophotometer (AAS). In addition, the pH of each sample is measuring to determine the correlation between the zinc dissolution and pH (Goteti *et al.* 2013). The amount of solubilization percentage = amount solubilized/total amount × 100% (Murphy and Riley 1962).

VI. THE ROOT COLONIZATION BY ZSB

After inoculation with bacteria and plant culture, it is necessary to determine root colonization by bacteria. To confirm the root colonization by bacteria can be used SEM. For this, after 5 – 7 weeks after plant cultivation, the root of the plant is sampled and operated as described by Adediran *et al.* (2015). The presence of bacteria at the root surface of the plant indicates successful colonization of the root by inoculated bacteria, if the soil and water used for the experiment have been sterilized, previously. To start root colonization by microbes, the plant needs to secrete compounds, root exudates, into the rhizosphere. For example, the rice plant uses the production and secretion of glucose to the rhizosphere to attract beneficial microbes to the roots. Here glucose acts as a ZSB chemoattractant to the microbes for binding the roots. ZSB colonized plant roots act as an upgraded root to absorbing zinc. Therefore, root colonization with zinc-soluble bacteria (ZSB) and fungi (ZSF) is of special importance for the plant. There are reports that the roots of plants inoculated with ZSB have a better growth status due to higher uptake of zinc during plant growth (Vaid *et al.*, 2014). It has been reported that inoculation of rice plant with *Bacillus* sp. bacterium as ZSB improved grain yield and growth parameters of Indian rice plant, it was shown that this ZSB increased the yield of two rice species (basmati-385 and super basmati) by 22% and 47%, respectively. In this study, ZSB effectively colonized the roots of the rice plant (Shakeel *et al.*, 2015). Inoculation of the plant with *Acinetobacter* sp. (TM56) with 0.2 mg/l zinc sulfate resulted in the best plant and root growth compared to the control treatment. In conclusion, the ZSB inoculation were able to colonize in the plant roots and enhanced their growth and development.

ZSB as a Biofertilizers

The use of Zn chemical fertilizer meets the needs of plants, but in the other hand, about 96-99% of applied Zn is converted to various insoluble forms based on soil type and physicochemical reactions (Saravanan *et al.* 2004). In the meantime, there are microbes that, by settling in the rhizosphere, show the ability to increase the solubility and bioavailability of Zn and reduce and eliminate the symptoms of zinc deficiency in the plant. Various rhizobacteria, such as the genera *Bacillus* and *Pseudomonas*, have been using developed mechanisms such as the production of protons, chelating ligands, and oxido-reductive systems at the cellular and membrane levels to dissolve metals such as Zn complexes. In addition to increasing the availability of nutrients for the plant, these beneficial bacteria have other beneficial traits for stimulating its growth, including the production of phytohormones, antibiotics, siderophores, vitamins, antifungals metabolites and hydrogen cyanide. Studies have shown that the use of *Bacillus* sp., a ZSB strain, as a Zn biofertilizer combined with the use of cheap and insoluble-zinc sources such as zinc oxide (ZnO), zinc carbonate (ZnCO₃), and zinc sulfide (ZnS), leads to increased zinc uptake by the plant and is therefore recommended as a suitable alternative to the expensive chemical fertilizer Zn sulfate (Mahdi *et al.* 2010 a,b). Various studies have shown that the superior strains of ZSB are distributed among different genera of PGPR, and also different PGPR characteristics have been reported among ZSB strain. A ZSB may have one or more PGP features. Indigenous soil rhizobacteria can partially affect the ability of strains used in biofertilizers. Therefore, to estimate the soil response to the PGPRs inoculation, it is necessary to have information about the history of PGPR and their performance in soil. Features reported for ZSB used in biofertilizers include increasing the bioavailability of nutrients such as phosphorus, potassium, zinc, iron, etc., development of plant growth by secretion of phytohormones such as auxin, production of siderophore for iron nutrition and antipathogenic activity, production of various antibiotics, also reducing the ethylene levels with the enzyme ACC deaminase under stressful conditions.

There have been reports of increased productivity of chemical fertilizers when combined with biofertilizers. Different bacterial genera including *Pseudomonas*, *Bacillus*, *Azospirillum*, *Enterobacter*, *Klebsiella*, *Rhizobium*, *Burkholderia*, and *Azotobacter* have been shown to enhance plant growth and development. These PGPRs were used as biofertilizers and increased growth by a variety of mechanisms, including solubilizing of insoluble Zn containing sources and increased its bioavailability for the plants. The biofertilizers, unlike chemical fertilizers, improve the soil fertility and accelerate its regeneration. The use of biofertilizers containing ZSB has been reported to increase plant growth and stability, improve grain yield, reduce malnutrition, and reduce dependence on chemical fertilizers (Huffes *et al.* 2001). One basic way to reduce the damage caused by chemical inputs to the

environment is to use ZSB along with other chemical fertilizers, which will be a major advantage for the use of fertilizers application (Zaidi and Mohammad 2006; Gul *et al.* 2004). The biofertilizer containing the bacterium *Bacillus aryabhatai* isolated from soybean rhizosphere grown in the vertisols of central India has been reported to have good ability to solubilize the insoluble Zn and increase plant growth indicators. The research shows that

ZSB has a positive effect on root dry matter, beard length, root volume, root surface and index of panicle emergence. He *et al.* (2010) reported that plant inoculation with ZSB, especially *Bacillus* sp., led to a maximum increase in plant growth parameters. Similarly, another study reported that zinc uptake and accumulation of dry matter increased through inoculation with PGPR-ZSB (Rana *et al.* 2012).

VII. FUTURE PERSPECTIVE

The strategy to solving the problem of zinc deficiency in the soil will eliminate zinc deficiency in plants and consequently human's body. Farmers should be aware that they can overcome the problems of plant nutrient deficiencies, including zinc deficiency, by using biofertilizers. It can also help them to reduce the cost of expensive micronutrient fertilizers. In order to have a healthy agriculture, a healthy environment and a healthy human being, we have to take steps to use these fertilizers. The use of native ZSB for non-native products may have conflicting results, but this problem can be overcome with further laboratory studies. For example, the use of microbial strains with the ability to produce spores can help to fertilize microbial survive in harsh soil conditions, or the use of multi microbial strains instead of one. The long-term use of biofertilizers not only promotes the health of agricultural products, soil and the environment is also economically viable. Today, with the increase of knowledge and awareness about beneficial soil microorganisms, the identification of beneficial microbes with multiple traits to stimulate plant growth such as atmospheric nitrogen fixation, dissolution of insoluble phosphate and potassium, production of various phytohormones, production of siderophore, etc., has accelerated. To complete the optimal and practical use of ZSB as an effective biofertilizer, the intervention of new technologies such as genetic alteration and modification is also necessary for these beneficial microbes to have good performance in soil and rhizosphere. The best suggestion for producing biofertilizers for agriculture in an area is to use native microbes that are fully compatible with that region. To develop microbial diversity, there must be a search for new and efficient species of ZSB as Zn biofertilizers for each region. Many recent studies show a promising trend of simultaneous inoculation of bacterial strains with beneficial synergies in the field of inoculation technology. It is better to isolate microbial strains from different soil and plant roots that be resistant to stress conditions. ZSB microbial strains that can competing ability, controlling and eliminating plant pathogens should also be given priority in research. In order to easily understand the mechanisms of zinc uptake and transmission in plants, genetic studies of ZSB strains and molecular properties of plant components as well as the plant-microbe interactions are essential. Recently, studies have focused on the combined use of microbial strains with different traits of nitrogen fixation, phosphate and potassium dissolution, iron supply, production of phytohormones, and antagonistic activity to achieve synergistic trend between these microbes to improve the biofertilizers performance. To achieve such a biofertilizer consisting of multiple microbial strains with effective performance, relevant scientists must overcome the following problems: 1- How to combine different microbes so that no negative and antagonistic effects are observed between microbes. 2- A suitable carrier that can be used for a wide range of microbes and supports the survival of various microbes. 3- The best and most practical way to establishment a microbial consortium in the soil and around the roots.

VIII. CONCLUSIONS

Zinc as an essential element for plants, which is vital for humans and animals. Insufficient knowledge and information of farmers about the causes of zinc deficiency in the soil has led to the emergence of malnutrition in more than half of the world's population. Chemical fertilizers are used to solve this problem, which leads to high costs, environmental pollution and loss the fertility of the soil in the long run. However, to overcome Zinc deficiency in soil and plants, it is possible to benefit the potential of beneficial soil rhizobacteria especially Zinc solubilizing bacteria (ZSB) by mechanisms such as proton production, organic acids, as well as

the secretion of siderophores that can absorbable the zinc for deficient plants. ZSB not only inhibit the development of zinc deficiency, but also improve the uptake of other macronutrients and micronutrients such as phosphorus, nitrogen, potassium and iron, as well as they can sometimes control plant pathogens, ultimately leading to higher performance and quality in agricultural product. Therefore, it is important to better understand the interaction between plants and microbes. This approach is effective for plant growth, soil health and soil fertility, and sustainable agriculture, and is promising because of economical and eco-friendly nature.

Conflict of interest

The authors declare that they have no conflict of interests

REFERENCES

- [1]. Adediran, G.A., Ngwenya, B.T., Mosselmans, J.F.W., Heal, K.V. and Harvie, B.A., 2015. Mechanisms behind bacteria induced plant growth promotion and Zn accumulation in Brassica juncea. *Journal of hazardous materials*, 283, pp.490-499.
- [2]. Altomare, C., Norvell, W.A., Björkman, T. and Harman, G.E., 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295- 22. *Appl. Environ. Microbiol.*, 65(7), pp.2926-2933.
- [3]. Anitha, S. and SN, P.D., 2015. Isolation and identification of zinc solubilizing fungal isolates from agricultural fields. *The Indian Journal of Agricultural Sciences*, 85(12), 1638-1642.
- [4]. Di Simone, C.D., Sayer, J.A. and Gadd, G.M., 1998. Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. *Biology and Fertility of Soils*, 28(1), pp.87-94.
- [5]. Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y.K., Subila, K.P. and Aravind, R., 2015. Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. *Microbiological research*, 173, 34-43.
- [6]. Fasim, F., Ahmed, N., Parsons, R. and Gadd, G.M., 2002. Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS microbiology letters*, 213(1), pp.1-6.
- [7]. Fomina, M., Alexander, L.J., Hillier, S. and Gadd, G.M., 2004. Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. *Geomicrobiology Journal*, 21(5), pp.351-366.
- [8]. Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*, 41(2), pp.109-117.
- [9]. Gontia-Mishra, I., Sapre, S. and Tiwari, S., 2017. Zinc solubilizing bacteria from the rhizosphere of rice as prospective modulator of zinc biofortification in rice. *Rhizosphere*, 3, 185-190.
- [10]. Goteti, P.K., Emmanuel, L.D.A., Desai, S. and Shaik, M.H.A., 2013. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). *International journal of microbiology*, 2013.
- [11]. Gregory, P. J., Wahbi, A., Adu-Gyamfi, J., Heiling, M., Gruber, R., Joy, E. J., and Broadley, M. R. (2017). Approaches to reduce zinc and iron deficits in food systems. *Global Food Security*, 15, 1-10.
- [12]. Hussein, K.A. and Joo, J.H., 2017. Stimulation, purification, and chemical characterization of siderophores produced by the rhizospheric bacterial strain *Pseudomonas putida*. *Rhizosphere*, 4, 16-21.
- [13]. Idayu, O., Maizatun, N., Radziah, O., Halimi, M.S. and Edaroyati, M.W., 2017. Inoculation of zincsolubilizing bacteria with different zinc sources and rates for improved growth and zinc uptake in rice. *International Journal of Agriculture and Biology*, 19(5), 1137-1140.
- [14]. Joy, E.J., Ander, E.L., Young, S.D., Black, C.R., Watts, M.J., Chilimba, A.D., Chilima, B., Siyame, E.W., Kalimbira, A.A., Hurst, R. and Fairweather- Tait, S.J., 2014. Dietary mineral supplies in Africa. *Physiologia Plantarum*, 151(3), pp.208-229.
- [15]. Kumar, A.S., Meenakumari, K.S. and Anith, K.N., 2017. Screening for Zn solubilisation potential of soil bacteria from Zn deficient soils of Kerala. *Journal of Tropical Agriculture*, 54(2), p.194.c
- [16]. Production of siderophores by the bacterium *Kosakonia radicincitans* and its application to control of phytopathogenic fungi. *Bioresource Technology Reports*, 3, pp.82-87.
- [17]. Lindsay, W.L. 1979. *Chemical Equilibrium in Soils*. John Wiley and Sons, New York.
- [18]. Madhaiyan, M., Saravanan, V.S., Jovi, D.B.S.S., Lee, H., Thenmozhi, R., Hari, K. and Sa, T., 2004. Occurrence of *Gluconacetobacter diazotrophicus* in tropical and subtropical plants of Western Ghats, India. *Microbiological research*, 159(3), pp.233-243.
- [19]. Marschner, P., Crowley, D., and Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—model and research methods. *Soil Biology and Biochemistry*, 43(5), 883-894.
- [20]. Masalha, J., Kosegarten, H., Elmaci, Ö. and Mengel, K., 2000. The central role of microbial activity for iron acquisition in maize and sunflower. *Biology and Fertility of Soils*, 30(5-6), 433-439.
- [21]. Nahas, E., 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World Journal of Microbiology and Biotechnology*, 12(6), pp.567-572.
- [22]. Nguyen-Deroche, T.L.N., Caruso, A., Le, T.T., Bui, T.V., Schoefs, B., Tremblin, G. and Morant-Manceau, A., 2012. Zinc affects differently growth, photosynthesis, antioxidant enzyme activities and phytochelatin synthase expression of four marine diatoms. *The scientific world journal*, 2012.
- [23]. Othman, N.M.I., Othman, R., Saud, H.M. and Wahab, P.E.M., 2017. Effects of root colonization by zincsolubilizing bacteria on rice plant (*Oryza sativa* MR219) growth. *Agriculture and Natural Resources*, 51(6), pp.532-537.
- [24]. Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L.S. and Fernández, F.J., 2007. OCAS, a fast and universal method for siderophore detection. *Journal of microbiological methods*, 70(1), 127-131.
- [25]. Rani N., Kaur R. and Kaur S. 2020. Zinc solubilizing bacteria to augment Soil Fertility – A Comprehensive. *IJASVM*. 8 (1), pp.38-44.
- [26]. Rengel, Z. (2015). Availability of Mn, Zn and Fe in the rhizosphere. *Journal of soil science and plant nutrition*, 15(2), 397-409.
- [27]. Saharan, B.S. and Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci MedRes*, 21(1), 30.
- [28]. Saravanan, V.S., Kalaiarasan, P., Madhaiyan, M. and Thangaraju, M., 2007. Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn²⁺) and zinc chelates on root knot nematode *Meloidogyne incognita*. *Letters in applied microbiology*, 44(3), pp.235-241.
- [29]. Saravanan, V.S., Madhaiyan, M. and Thangaraju, M., 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere*, 66(9), pp.1794-1798.
- [30]. Saravanan, V.S., Subramoniam, S.R. and Raj, S.A., 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian Journal of Microbiology*, 35(1-2), pp.121- 125.

- [31]. Sarikhani, M.R., Khoshru, B. and Oustan, S., 2016. Efficiency of some bacterial strains in potassium release from mica and phosphate solubilization under in vitro conditions. *Geomicrobiology journal*, 33(9), 832-838.
- [32]. Shakeel, M., Rais, A., Hassan, M.N. and Hafeez, F.Y., 2015. Root associated *Bacillus* sp. Improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. *Frontiers in microbiology*, 6, p.1286.
- [33]. Sharma, A., Johri, B.N., Sharma, A.K. and Glick, B.R., 2003. Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L.). *Soil Biology and Biochemistry*, 35(7), 887-894.
- [34]. Sharma, P., Kumawat, K.C., Kaur, S. and Kaur, N., 2014. Assessment of zinc solubilization by endophytic bacteria in legume rhizosphere. *Indian J Appl Res*, 4(6), pp.439-441.
- [35]. Snell F. D and Snell C. T., 2007. *Colorimetric Methods of Analysis* 7 th Ed., Van Nostrand, Princeton.1245.
- [36]. Vaid, S.K., Kumar, B., Sharma, A., Shukla, A.K. and Srivastava, P.C., 2014. Effect of Zn solubilizing bacteria on growth promotion and Zn nutrition of rice. *Journal of soil science and plant nutrition*, 14(4), 889-910.
- [37]. Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. and Bashan, Y., 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils*, 30(5-6), pp.460-468.
- [38]. Vidyashree, D.N., Muthuraju, R., Panneerselvam, P. and Mitra, D., 2018. Organic Acids Production by Zinc Solubilizing Bacterial Isolates. *Int. J. Curr. Microbiol. App. Sci*, 7(10), pp.626-633.
- [39]. Wakatsuki, T., 1995. Metal oxidoreduction by microbial cells. *Journal of industrial microbiology*, 14(2), pp.169-177.
- [40]. White, C., Sayer, J.A., Gadd, G.M. 1997. Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS microbiology reviews*, 20(3-4), pp.503-516.
- [41]. Zhu, H., Yang, H. 2015. Isolation and characterization of a highly siderophore producing *Bacillus subtilis* strain. In: *Advances in Applied Biotechnology*. Springer Berlin Heidelberg. 83-90.
- [42]. Alloway, B. J. (2008). Zinc in soils and crop nutrition, pp.19.
- [43]. Vodyanitskii, Y. N. (2010). Zinc forms in soils (Review of publications). *Eurasian Soil Science*, 43(3), 269-277.