

Applications of Microorganisms in Agriculture for Nutrients Availability

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Abstract

Constantly increasing global food demand, over use of Phosphorus (P) containing fertilisers to improve agricultural productivity not only causes pollution of ground and surface water but also depletes soil fertility and leads to accumulation of toxic elements in the soil. Production of healthy agriculture crops is essentially dependant on the available nutrients in soil. In healthy crop production, key nutrients such as phosphorus (P), iron (Fe), nitrogen (N) and potassium (K) play a major role. Chemical fertilizers are in great demand as most soils are deficient in key nutrients. Hence efforts are made for an innovative, alternative and environmentally friendly techniques over conventional chemical fertilizers. Microbes play a pivotal role in increasing the nutrients bioavailability such as mobilization of major nutrients and nitrogen fixation that improve the plant yield and growth. In this review, current findings on the mechanisms utilized by various microbes will be discussed and highlighted. Furthermore, in improving soil fertility the inoculation of microbes in various forms such as sole inoculation, co-inoculation or in combination with organic fertilizer will be debated.

Keywords: Soil depletion, soil microbes, solubilization, co-inoculation, biofertilizer

1.1 Introduction

In developing countries from 1970 to 1995, the global food production had increased by 70% mainly because of new technology called conventional agriculture which was based on chemical fertilizers, synthetic pesticides and irrigation to produce high yields [1, 2]. The use of such technologies are destroying the natural environment in many ways such as surface and ground water contamination, increased pest resistance, soil erosion and reduced biodiversity [3, 4]. Keeping in mind the increasing population and food demand worldwide, sustainable methods are key for food production. To avoid mitigating climate change and land degradation the sustainable methods must sustain this natural resource. This can be done by fine tuning of current technologies or to develop alternate technologies without

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damaging the environment or natural resources to obtain high yield crops that can meet food demand globally.

About organic and inorganic fertilizers there are numerous myths [5, 6]. Without enough scientific evidence, it appears that mostly these myths are based on conjunctions, perceptions, or for political motives. Regarding chemical fertilizers some legends are listed below:

1.1.1 Land and Soil Deterioration

Soil's physical and chemical properties are destroyed by chemical fertilizers whereas fertility is improved by organic fertilizers [5]. Soil structure by chemical fertilisers is not affected if it is administered in the right quantity. Nitrogen (N) fertilizers tend to acidify soil to a pH of < 5 which can have adverse effects. If administered at an optimal dose it may positively influence soil biota. If applied in high amount then it may alter soil texture, reduce in microbial community and increase soil acidification.

1.1.2 Micro-Nutrients Lacks

Regarding chemical fertilizers there is a popular misconception that macro-nutrients are in low amounts when in fact they are lacking. Organic fertilizers generally contain some micro-nutrients however there are some inorganic fertilizers containing micro-nutrients commercially available too [5].

Adoption of Integrated Soil Fertility management can help restore fertility of soil as explained by Vlek and Vielhauer [7]. This involves a strategy that allows for control of soil nutrients, nitrogen fixation and efficiency in administration.

This strategy encompasses use of biofertilizers which are biocompatible and economically viable plant nutrients to help supplement synthetic fertilisers for a more sustainable agricultural system. The aim is to increase production and sustain balance of the nutrients in soil.

1.2 Biofertilizers

Microbial inoculants or biofertilizers are live or latent cell of strains that are efficient in phosphate solubilization, nitrogen fixation, potassium solubilization, siderophore production used for seed application, soil or composting areas where such microorganism population can increase and enhance several microbial processes to boost the nutrients availability that can be utilised by plants. The history of applying microbial inoculums have passed down generations of farmers. The efficacy of biofertilizers was evident when on small scale culture compost was introduced, it enhanced the decomposition of organic residues and agriculture by-products that resulted in healthy harvest of crops [8].

In biofertilizer making several things are considered such as growth profile of the selected microbe, organism's optimum conditions and inoculum formulation. For the success of biological product critical steps are inocula formulation, application method and product storage.

Generally, six key steps are required in making a biofertilizer, 1) organism selection, 2) solation, 3) method selection and carrier material, 4) propagation method selection,

5) prototype testing and 6) large scale testing. In late 1940's, Malaysia started using industrial scale microbial inoculants and took guide by Brady rhizobium on legumes topped up in 1970's. Malaysian Rubber Board (MRB), a government research institute has conducted research on Rhizobium inoculums for use on leguminous cover crops of young rubber trees in the large-scale plantations. Since 1980 many research projects on Mycorrhiza were conducted at University Putra Malaysia (UPM) and led to Azospirillum nitrogen to oil palm seedlings [8].

1.3 Rhizosphere

Root network is surrounded by a narrow zone of soil termed as rhizosphere [9], and the bacteria that colonises in the root environment are termed 'rhizobacteria' [10]. These roots not only provide mechanical support, nutrient uptake and facilitate water but also synthesize various compounds [9]. Heterogeneous, multifaceted and active microbial communities are attracted to the compounds produced by roots. In a nutshell they act as chemical attractants commonly called as root exudates. The chemical and physical properties of soil are modified by wide variety of chemical compounds (root exudate) and thus control the structure of the microbial community found in the proximate area of root surface [11]. Some microorganisms are attracted whereas some are repelled by the exudates. Exudate composition depends upon plant species, physiological state and surrounding microbes [12]. In addition, these exudates enhance symbiotic plant interaction and growth inhibition to the hostile plant species [13, 14]. In the rhizosphere microbial activity also affects nutrient availability and root patterns thereby by modifying the quantity as well as quality of root exudates. Small organic molecule fractions derived by plants are metabolized further as nitrogen and carbon sources by microbes present in the vicinity. On the other hand, molecules obtained from microbes are regained by plants for growth and development [12]. Critical determinants of rhizosphere function are carbon fluxes. 5 to 15 percent of fixed carbon is obtained through root exudation and transported to rhizosphere [15]. Any amount of soil associated or influenced by plants roots or their hairs as well as any plant produced materials encompass the rhizosphere [16]. Basically, rhizosphere can be grouped into three but interacting constituents a) rhizosphere (soil), b) rhizoplane (root surface), and c) root (itself). Rhizosphere is an area of soil which affects the microbial activity through the release of substrates by roots whereas rhizoplane is the root surface covered by soil particles and roots by microbes known as root colonization [17].

1.4 Plant Growth Promoting Bacteria

Plant growth promoting rhizobacteria (PGPR) are bacteria that colonise the rhizosphere. When PGPR are introduced to roots, seeds, or into soil either directly or indirectly they stimulate plant growth. They play an essential role in plant growth by increasing nutrient uptake, suppress plant pathogens and induce plant resistance to pathogens. The term PGPR was first coined by Kloepper and co-worker's in the late 1970s [18]. PGPR are found widely in various genera such as *Azospirillum*, *Agrobacterium*, *Acinetobacter*, *Arthrobacter*, *Bradyrhizobium*, *Bacillus*, *Burkholderia*, *Pantoea*, *Cellulomonas*, *Frankia*, *Rhizobium*,

Thiobacillus, *Pseudomonas*, *Serratia*, *Streptomyces*. Rhizobacteria are most widely studied PGPR that colonizes the root surfaces and the rhizosphere [18, 19]. Some of these PGPR also enter root interior and establish endophytic populations as reviewed recently by Gray and Smith [19, 20].

Many of them can surpass the endodermis barrier and enter the vascular system by crossing the root cortex and bloom as endophytes in stem, tubers, leave and other parts [19, 21–23]. Endophytic colonization is characteristic of PGPR (the attribute of selective adaptation to specific ecological niches) [19, 21], as a result friendly association between the host plant and bacteria is formed [21, 23] excluding any damage to the host plants [24–26].

1.4.1 Nitrogen Fixation

For plant growth and productivity nitrogen is one of the most important nutrients. Atmosphere is composed of 78% nitrogen but still not available to plants. Plants can only utilise atmospheric nitrogen when it is converted to ammonia, easily taken by plants by the process known as nitrogen fixation [27]. Enzyme nitrogenase produced by nitrogen fixing microorganisms converts the atmospheric nitrogen to ammonia [28, 29]. These nitrogen fixing microbes have symbiotic and non-symbiotic relationship with the plants. Members of Rhizobiaceae family have a symbiotic relationship with leguminous plants [30]. Free-living and endophytic forms of microorganisms are reported non symbiotic such as *Azotobacter* *Cyanobacteria*, *Azospirillum* [31].

As stated earlier symbiotic rhizobium nitrogen fixing rhizobacteria belong to family Rhizobiaceae (α -proteobacteria) and they create a symbiotic relation by infecting the roots of leguminous plants leading to a formation of a nodule. Rhizobia resides in the nodule as an intracellular symbiont [32]. *Mesorhizobium*, *Sinorhizobium*, *Rhizobium*, *Azorhizobium* and *Bradyrhizobium*, are together termed as Rhizobia. Diazotrophs, are non symbiont rhizobacteria that fix nitrogen in non leguminous plants and have a non-obligate relation with the host plants [33]. Table 1.1, shows the most commonly studied bacteria for nitrogen fixation. The process of nitrogen fixation is carried out by an enzyme nitrogenase that carries out the process of nitrogen fixation. The complex enzyme structure consists of di nitrogenase reductase having iron as its cofactor and di nitrogenase having iron and molybdenum (Mo) as its cofactor [29]. Mo-nitrogenase, V-nitrogenase, and Fe nitrogenase are three different reported nitrogen complexes based on the variation in the cofactor di nitrogenase [34, 35]. Nif genes carry out Nitrogen fixation in both symbiotic and free-living nitrogen-fixing microorganisms [36]. Formation and function of the enzyme require electron donation, iron protein activation, biosynthesis, Fe-Mo cofactor and regulatory

Table 1.1 Most common bacteria studied for nitrogen fixation.

Kinds	Bacteria
Symbiotic	<i>Mesorhizobium</i> , <i>Sinorhizobium</i> , <i>Rhizobium</i> , <i>Azorhizobium</i> and <i>Bradyrhizobium</i>
Non-symbiotic	<i>Azotobacter</i> , <i>Beijerinckia</i> , and <i>Clostridium</i>

genes by Nif genes [37]. For nif gene expression oxygen is a negative regulator required for *Rhizobium sp.* bacteroid respiration [38]. The enzyme is kept active from oxygen as Bacterial leg haemoglobin with high affinity for oxygen. To pursue the process of nitrogen fixation efficiently oxygen supply to the bacteroid (for respiration) and nitrogenase enzyme complex prevention to oxygen should take place at the same time. To accomplish this task, simple way is to introduce bacterial haemoglobin through genetic engineering that binds the oxygen to the rhizobacteria [39]. This method was carried out through transformation by inserting haemoglobin gene of gram negative bacterium *Vitreoscilla sp.* to *Rhizobium etli*. Respiratory rate in rhizobial cells was two- to threefold higher as compared to the normal rhizobial cells [40]. Introducing the genes that produce haemoglobin from *Vitreoscilla sp.* to rhizobial cells started producing haemoglobin. So produced haemoglobin is transformed in the cell despite the low availability of oxygen which causes a strong affinity to oxygen. In bean plants significant 68% nitrogenase activity was observed when inoculated with transform rhizobium that showed leaf content was increased to 25-30% and 16% increase in the nitrogen content of the seeds [41].

It was reported that after the infection of legumes by *Rhizobium sp.* plants ethylene level was increased, and this increased ethylene concentration which helps in inhibiting the rhizobial infection and formation of nodules [42]. On roots of host legumes plant number of nodules formation can be increased by specific strains of rhizobia by synthesis of a small molecule termed "rhizobitoxine" that limit the increase in ethylene production [43]. It is a phytotoxin acts on the enzyme 1-aminocyclopropane-1-carboxylate (ACC) synthase that synthesizes ethylene [44]. ACC deaminase enzyme production has been reported by few rhizobial strains which removes some immediate precursor in plants before it translated to ethylene. The decrease in ethylene production leads to increase 25–40% biomass of the plant and nodule formation [45]. Naturally about 1-10% of rhizobial strain possess ACC deaminase and those strain that lack ACC deaminase genes, can gain this gene by introducing it into the strain through genetic engineering [46]. The insertion of an deaminase ACC gene has been reported from *Rhizobium leguminosarum bv.viciae* into *Sinorhizobium meliloti* chromosomal DNA, 35% nodule number dramatically increased and 40% biomass alfalfa host plants as compared to control wild type strain [41, 46].

Azorhizobium, another strain of Rhizobia that not only forms nodules in stem and fixes nitrogen but also increases production of IAA indole acetic acid [47]. Another efficient nitrogen fixer strain *Bradyrhizobium* when introduced in mucuna seeds results in increase nitrogen, total organic carbon, phosphorus and potassium contents in the soil. Hence increases plant biomass, growth, microbial population etc [48]. Non nodule-forming aerobic nitrogen-fixing bacteria *Azospirillum* from *Spirillaceae* family are gram-negative [49]. *Azospirillum halopraeferens*, *Azospirillum amazonense*, and *Azospirillum brasilense* are species from the same family whereas *A. brasilense* and *A. lipoferum* are major beneficial species [50]. Those plants having C4 dicarboxylic pathway (Hatch-Slack pathway) of photosynthesis *Azospirillum* form associative symbiosis with them as they use organic salts of malic and aspartic acid for their growth and nitrogen fixation [51].

Azotobacter belong to *Azotobacteriaceae* family, are free-living, aerobic, photoautotrophic. They are found usually alkaline and neutral soil. Within this family *Azotobacter chroococcum* are the most common species [52]. Other species include, *Azotobacter insignis*, *Azotobacter vinelandii*, *Azotobacter macrocytogenes*, *Azotobacter beijerinckii* [50]. They secrete various phytochromes such as naphthalene acetic acid, gibberellins, vitamin B

complex and other substances that not only promote uptake of minerals, root growth but also inhibit certain root pathogens [53]. *Azotobacter* secretes special substance which also improves root growth, uptake of plants nutrients however it also prevents growth of specific root pathogens [54, 55]. Antifungal antibiotics are produced by *Azotobacter indicum* another strain of *Azotobacter* inhibit the growth of few pathogenic fungi in the root region hence reduces the seed mortality [56]. In uncultivated soils and in rhizosphere zone of the crop plants *Azotobacter* population is generally very low and found in rhizosphere of several crop plants such as sugarcane, rice, bajra and vegetables [57]. Biofertilizers rich in *Azolla* provides nitrogen efficiently to rice plants as it decomposes in soil easily. In addition it plays a very important role in providing major amount of iron, zinc, phosphorus, molybdenum, potassium and other micronutrients [58]. Prior to rice cultivation *Azolla* was used a green biofertilizer. In India most commonly used is *Azolla pinnata* and it can propagate by vegetative means on large commercial scale [59]. *Azolla microphylla*, *Azolla caroliniana*, *Azolla filiculoides* and *Azolla mexicana* species of *Azolla* are used in India for their large biomass production [50].

1.4.2 Phosphate Solubilization

Event though a large amount of Phosphorus is present in soil however its availability to plants especially crops is low [60]. The low availability of phosphorus due to its insoluble forms and is only absorbed by plants in two forms which are monobasic and the di basic ions [31]. In chemical fertilizers much of the soluble inorganic phosphorus becomes immobilized after application to the field. So, it becomes unavailable to plants and hence it is wasted [61].

Most bacteria found in the rhizosphere are phosphate solubilising, they produce organic acids and phosphatases which helps in the conversion of insoluble phosphorus to crop available forms. The nutrients availability is increased by phosphate solubilization in host plant by PGPR (most common mode of action) present in rhizosphere [62]. Successful inoculants strains are *Rhizobium leguminosarum* bv. *Phaseoli* with maize, for wheat *Azotobacter chroococcum*, *B. circulans* and *Cladosporium herbarum*, *Enterobacter agglomerans* with tomato, *Pseudomonas chlororaphis* and *P. putida* with soybean, *Rhizobium* sp. and *Bradyrhizobium japonicum* with radish, *Falvobacterium*, *Microbacterium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Beijerinckia*, *Erwinia*, and *Serratia* [31, 63–68]. In soils and plant rhizosphere these zones particularly are rich in phosphate solubilizing bacteria includes both anaerobic and aerobic strains. In submerged soil aerobic strains are commonly found. Youssef and Eissa [49] reported that phosphate solubilising bacteria are not generally found in non rhizospheric zone compared to rhizospheric zone. The phosphate solubilization characteristic of rhizospheric bacterium by no means proves that it will function as a PGPR. Study of Cattelan *et al.* [68] showed that phosphate solubilisation was observed in five isolates but only two promoted seedling growth. Similarly plant growth is also not increased by all phosphate solubilising PGPR. de Freitas *et al.* [68] isolated *Bacillus* sp. and from *Canola* rhizosphere *Xanthomonas maltophilia* (*Brassica napus* L.) positive for phosphate solubilization no change in phosphate content of the host plant though increase effect on plant growth was observed. Environmental factors (such as stress conditions) affects establishment and performances of the phosphate solubilising bacteria [30].

1.5 Microbial Mechanisms of Phosphate Solubilization

1.5.1 Organic Phosphate

Organic acids, protons, siderophores, carbon dioxide and hydroxyl ion production are the principal mechanisms observed by phosphate microbial organisms [69]. Upon chelation of cations with carboxyl and hydroxyl ions or pH reduction causes organic acid production that releases phosphate [70]; periplasmic space is the place where organic acids are produced by the direct oxidation pathway [71].

1.5.1.1 Organic Acid Production

With the drop in pH, organic acids are secreted, the surroundings and the microbial cells are acidified that results in the release of phosphate by switching H^+ for Ca_2^+ [72]. Whereas it was observed that there is no direct correlation among amount of phosphate solubilisation and pH [73]. Therefore, the theory of H^+ acidification was proposed by Illmer and Schinner, 1995 [74]. They found cation assimilation releases H^+ . Phosphate solubilisation is carried out by the assimilation of NH_4 with H^+ [75]. With the help of H^+ translocation ATPase, H^+ are released in the outer surface in exchange for cation uptake results in the production of organic acid mineral phosphate solubilisation [69]. Studies also show that phosphate solubilization can be carried without any organic acid production, within the microbial cell's protons are release with the assimilation of NH_4^+ [75]. Among all the organic acid for phosphate solubilization gluconic acid is the most reported one, Figure 1.1, shows different types of acid produced by phosphate solubilising microbes The gluconic acid makes phosphate available to plants through chelation of cations bounded to phosphate. Phosphate is also solubilised by gram negative bacteria by direct oxidation of glucose to gluconic acid [76]. Pyrroloquinoline quinone (PQQ) in phosphate solubilization acts as a redox cofactor in glucose dehydrogenases (GDH) [77].

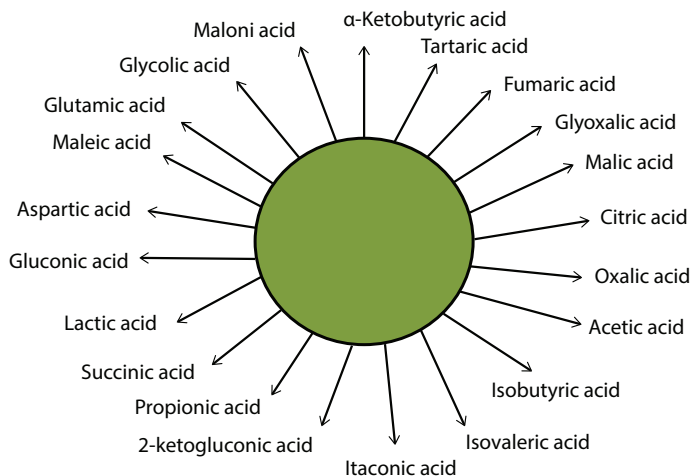


Figure 1.1 Various acid production by phosphate solubilising microbes. (Source: https://www.frontiersin.org/files/Articles/258916/fmicb-08-00971-HTML-r1/image_m/fmicb-08-00971-g002.jpg).

1.5.1.2 Inorganic Acid Production

Production of Inorganic acids (such as carbonic, nitric and sulphuric acids) and the production of chelating substances are also carried out by microorganisms for mineral phosphate solubilization. Although inorganic acid and chelating substance effectiveness is found to be less than that of organic acids. Kim *et al.* [78] studied that in culture medium the increase in phosphate concentration is not solely because of organic production. Moreover, it is also observed that plant roots are extended effectively by Mycorrhizal fungi, results in increase soil volume through which phosphate may be absorbed [79].

1.5.1.3 Enzymes Production

Another mechanism reported in the literature for microbial phosphate solubilization is the release of enzymes or enzymolysis. In an experiment mechanism of phosphate solubilization was studied lecithin containing medium. Increased acidity was observed by an enzyme that produced choline by acting on lecithin [80]. Moreover, in liberating phosphorus, esterase types enzymes are involved. Phosphatase enzymes are known to produce by phosphate solubilizing microbes that helps in phosphate solubilization along with acids in aquatic environment [81].

1.5.2 Organic Phosphate Solubilization

Organic matter is a significant and a major source of organic phosphorus in soil. Organic phosphate in soil is present as inositol phosphate also called as soil phytate. Xenobiotic phosphonates are found in large amounts to be in detergents additive, flame retardants, pesticides and antibiotics, and are released regularly into the environment containing organic phosphates. As a compound are resistant to chemical hydrolysis because of their high molecular weight so there is need for bioconversion either to soluble ionic phosphate (Pi , HPO_4^{2-} , H_2PO_4^-), or low molecular-weight organic phosphate, to be absorbed by microbial cells [82]. Halvorson *et al.* [83] proposed one important theory called as 'sink theory' for the solubilisation of organic phosphorus.

Phosphorus is continues removed by Ca-P compound dissolution. In the biomass of phosphate solubilizing microbes Phosphorus content is correlated to the decomposition of phosphorus in organic substrates [84]. Microbes play a vital role in phosphorus cycle; various enzymes are involved. Enzymes that dephosphorylate the phosphor-ester or phosphoanhydride bond of organic compounds, are non-specific acid phosphatases. Phosphomonoesterases is an example of non-specific acid phosphatases (NSAPs) is the most studied enzyme produced by phosphate solubilizing microbes [85]. These enzymes can be acid or alkaline in nature [86]. The soil pH ranges from acidic to neutral values mostly where phosphatase activities occur. In this process it shows that acid phosphatase plays a vital role [87]. In production of organic phosphorus, phytase is an enzyme produced by phosphate solubilizing microbes. Phytase releases phosphorus in soil from organic materials such as pollen and seeds of plants that are deposited in phytate form. This phytate is degraded by phytase enzyme and phosphorus is released and available for plant use. The phosphate solubilizing microbes play an important in the availability of phosphorus to plants as plants generally cannot take phosphorus directly from phytate [88].

1.6 Bacterial and Fungi Coinoculation

Integrated nutrient management strategy of degraded soils leads to potential use of bacterial and fungal inocula [89]. According to Ahemad and Kibret [37], by introduction of bacterial and fungal inocula we can explore potassium, phosphate, iron reserves, fix nitrogen from the environment and boost organic matter [90–92]. Out of the total nitrogen demand of grassland and savannah only 5–20% is fixed by biological N fixing bacteria and arbuscular mycorrhizal fungi [93]. Temperate and boreal forests fungi contribution is 80% whereas 75% of phosphate acquired by plants through fungi and bacteria. Bioavailability of nutrients by fungi and bacteria is mostly due the production of organic acids and siderophores. To promote soil aggregate stability process carried out by fungi and bacteria is also the excretion of organo-polysaccharides and proteins (hydrophobins, golmalin and mucilages) are also reported [91, 92, 94].

Bacteria and fungi are pervasive and are present in air, soil and water. They are also reported for soil aggregation and forming pores within to improve soil structure [95]. Fungal cells release mucilaginous exudates composed of extracellular surface polysaccharides. Intracellular polysaccharides and extracellular polysaccharides are found to be in the cytoplasmic membrane. Extracellular polysaccharides play a very significant role in soil aggregation that aids in porosity and aeration in soil. Whereas in bacteria exopolysaccharides are released when they form organo-mineral complexes that aids in soil aggregation to hold the soil particles together [95]. It has been reported structure of the soil is not made by mineral constituents but as the microorganisms resides in the soil helps in pores formation [96]. Soil structure, function and quality are improved by its organic matter that consist of plant and animal residues, decomposed cells and exudates from bacteria and fungi. Co-inoculation of fungi and bacteria with organic amendment may help with sustainable management of fertility with regard to soil and crop production [96]. There are still many undeveloped factors that need to be explored, in order to gain maximum benefit from such strategies.

1.7 Conclusion

A major threat to food security is land degradation in the coming decades. Agricultural land is degrading, and its viability is declining and as much as 84% is affected due to human activity making it harder to sustain crop production to feed the world. To restore degraded soils microorganisms especially the bacteria and fungi play a vital role. Using these microbes for degraded soil remediation could be a promising approach and may help us to prevent the wide use of chemical fertilizers. These microbes are capable of fixing nitrogen, acids, proteins and siderophores production resulting in increased nutrient bioavailability, soil quality and may help in reinstating the fertility of degraded soils. The incredible benefits which are achieved by these microbes, although exact mechanisms are not fully studied. To enhance soil fertility for crop production of degraded soil studies of mechanisms which are cost effective and innovative practice are required. Co-inoculation of bacteria and fungi with organic fertilisers can prevent unnecessary use of chemical fertilisers.

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