COMMON-USED BIOFERTILIZERS

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BIOFERTILIZERS: DEFINITION AND GENERAL ASPECTS

The increasing demand for safe and healthy food and the concerns on environmental pollution have led to the emergence and development of organic farming. It is globally an important priority area in the crop and livestock production, which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. Organic farming is based on the development and application of biofertilizers and plant strengtheners. The use of chemical fertilizers in large amounts has resulted in a manifold increase in the productivity of farm commodities but they also have an adverse effect on the soil. Continuous and excess use of chemical fertilizers and other agrochemicals to increase yield may lead to ground water contamination and depletion of soil nutrients, eventually resulting in reduction of crop yield. This problem could be overcome using a different technology to produce various biofertilizers. Biofertilizers from microorganisms can replace chemical fertilizers; they are less expensive and are more environmentally friendly than chemical fertilizers. The current global market for organically raised agricultural products is valued at around US$ 30 billion with a growth rate of around 8 percent. Nearly 22 million hectares of land are now cultivated organically. Organic cultivation represents less than 1 percent of the world's conventional agricultural production and about 9 percent of the total agricultural area. Biofertilizers, or more appropriately "microbial inoculants" in the strict sense, are not fertilizers, which directly give nutrition to crop plants. They represent natural and organic formulations that contain living or latent cells of beneficial soil microorganisms which, after being added to the seeds, plant surfaces or soil, colonize the rhizosphere or the interior of the plant and promote its growth by increasing the supply or availability of primary nutrients to the host plant. The inoculation with beneficial soil microorganisms is a promising method for raising soil fertility because, in this way, the accessibility of plants to a number of important elements, such as nitrogen, phosphorus and potassium, increases. As a result, the use of synthetic fertilizers can be significantly reduced. In the world literature, there is evidence of promotion of vegetable yields by inoculation with microorganisms. Microorganisms (bacteria, mycorrhizal fungi and algae) are the living components of the soil. Their activities related to soil fertility and plant nutrition are diverse. They affect the soil structure, the dynamics of nutrients in it, participate in plant nutrition and increase plant resistance to soil-borne pathogens.

These microorganisms are responsible for the process of nitrogen fixation, solubilization of insoluble soil phosphates, convertion of complex organic biomass into mineral compounds which are utilized by plants, and synthesis of growth-promoting substances such as amino acids, vitamins, etc. There are 17 essential non-mineral and mineral elements required for proper plant growth. The lack of any of these nutrients can result in severe damage to crop health. Three essential nutrients are carbon (C), hydrogen (H) and oxygen, which are taken up from atmospheric carbon dioxide and water. Of the mineral elements, the primary macronutrients (nitrogen, phosphorous, and potassium) are needed in largest quantities and are most likely to be in short supply in agricultural soils. Secondary macronutrients, such as Mg, S, Zn, Mn, Fe and Cu, are needed in smaller quantities and are typically found in sufficient quantities in agricultural soil, and therefore do not often limit crop growth. Micronutrients, or trace nutrients (B, Mo, Cl, and Ni) are needed in very small amounts and can be toxic to plants in excess. Silicon (Si) and sodium (Na) are sometimes considered essential plant nutrients, but due to their ubiquitous presence in soils, they are never in short supply. Microorganisms encourage plants to absorb a greater quantity of nutrients on their own which, even if naturally present in the soil, on occasion, cannot be assimilated by plants because of being in an insoluble form.

At present, biofertilizers are supplied to the farmers as carrier-based inoculants or as liquid formulations as an alternative technology, which has more advantages than the carrier-based inoculants.

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TYPES OF BIOFERTILIZERS ON THE BASIS OF BENEFICIAL MICROORGANISMS AND THEIR FUNCTIONS

Biofertilizers contain microorganisms that are able to activate a biological process which stimulates the development of plants and ensures healthy growth. These microorganisms do not function only as a fertilizer. They transform the inaccessible forms of soil elements into ones accessible to plants. Although they are called fertilizers, they do not contain all nutrients that may be added directly into the soil to increase soil fertility. On the contrary, microorganisms slowly and reliably improve the soil stability and phytosanitation. The difference between biofertilizes and composts lies in the amount of microorganisms contained in them. Biofertilizes can comprise only a specific strain of microorganism, which is intended for a specific activity in the soil. These microorganisms are classified into three main groups: nitrogen-fixing, phosphate-transforming and cellulose-degrading microorganisms. They help to fix atmospheric nitrogen and to convert the phosphorus into a form usable to plants.

Microorganisms also help plants to produce hormones, vitamins and amino acids that are of substantial importance for building resistance to pathogens. Almost all crops need different types of biofertilizers depending on their needs. The various types of biofertilizers which help plants grow at different phases of growth can be grouped into four categories:

- N-fixing biofertilizers: These include the bacteria *Rhizobium*, *Azotobacter*, *Azospirillum*, *Clostridium* and *Acetobacter* among others; blue-green algae (BGA), or cyanobacteria, and the fern *Azolla* (which works in symbiosis with BGA).
- P-solubilizing/mobilizing biofertilizers: These include phosphate-solubilizing bacteria (PSB) and phosphate-solubilizing microorganisms (PSMs) like *Bacillus*, *Pseudomonas* and *Aspergillus*. *Mycorrhizae* are nutrient-mobilizing fungi, also known as vesicular-arbuscular mycorrhizae, or VA-mycorrhizae or VAM.
- Plant-growth-promoting rhizobacteria (PGPR): Mainly represented by species of *Pseudomonas*. These bacteria do not provide plant nutrients but they enhance plant growth and performance.
- Composting accelerators: cellulolytic (*Trichoderma*) and lignolytic (*Humicola*) fungal species and different Gram-positive and Gram-negative bacteria.

Nitrogen-fixing biofertilizers

Nitrogen is the most limiting nutritional factor for plant growth. Suitable nitrogen application to growing plants has a favourable enhancing effect on growth, yield and quality. Since nitrogen is the main element in the composition of amino acids, which are required for the synthesis of proteins and other related compounds, it plays a role in almost all plant metabolic processes. Nitrogen is also an integral part of the chlorophyll molecule responsible for plant photosynthesis. Symptoms of nitrogen deficiency generally appear on the bottom leaves first; the lower leaves on the tips turn brown, usually disintegrate, and fall off. However, the excessive use of nitrogen fertilizers increases the total costs of crop production, pollutes the agro-ecosystem and enhances the deterioration of soil fertility. Therefore, it became essential for researchers to develop and adopt a strategy of supplementing or substituting inorganic nitrogen with organic sources, especially ones of microbial origin. Nitrogen-fixing biofertilizers were the ones majorly utilized in the industry in 2012, accounting for over 78% of the global demand. These biofertilizers are mainly used for crop yield improvement and involve several potential benefits in environmental application, in addition to their agricultural usefulness. Furthermore, increasing consumption of leguminous and non-leguminous plant products is also expected to augment the demand for nitrogen fixing biofertilizers over the forecast period.

Nitrogen biofertilizers help agriculturists to determine the nitrogen level in the soil. The type of crops also determines the level of nitrogen. Some crops need more nitrogen for their growth, while others need fewer amounts. The type of soil is an important factor which determines which type of biofertilizers is needed for a crop.

Though the atmospheres contain 79% N_2, eukaryotes cannot utilize it directly. Atmospheric N_2 must be first reduced to nitrogen compounds that can be assimilated by plants (either NH_4^+ or NO_3^-). This process is called biological nitrogen fixation (BNF) and is exclusively carried out by prokaryotes (bacteria and cyanobacteria) (Fig.1).

1. Uptake of NH_4 or NO_3 by organisms
2. Release of NH_4 by decomposition
3, 4. Microbial oxidation of NH_4 (yields energy in aerobic conditions)
5. Denitrification (NO_3 respiration) by microbes in anaerobic conditions (NO_3 is used as a terminal electron acceptor during decomposition of organic matter)
6. Nitrogen fixation

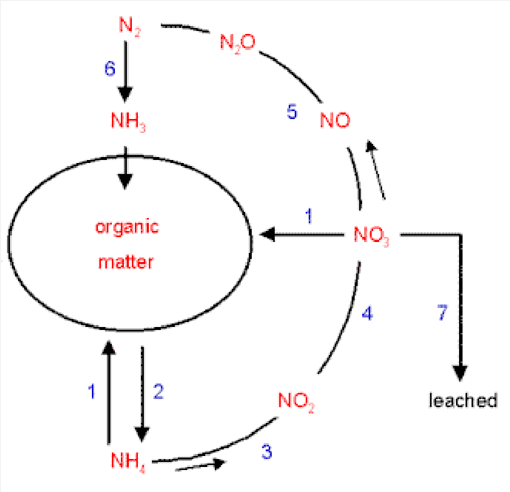

Fig. 1. Nitrogen cycle in nature

The diagram above shows an overview of the nitrogen cycle in soil or aquatic environments. At any time, a large proportion of the total fixed nitrogen will be locked up in the biomass or in the dead remains of organisms (shown collectively as "organic matter"). So, the only nitrogen available to support new growth will be that supplied by nitrogen fixation from the atmosphere (pathway 6 in the diagram) or by the release of ammonium or simple organic nitrogen compounds through the decomposition of organic matter (pathway 2).

Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck. It accounts for 60% of the total nitrogen fixation. The microorganisms that fix nitrogen are called diazotrophs.

In this way, they increase the soil nitrogen level and, respectively, the soil fertility. Biological nitrogen fixation is catalyzed by a microbial multimeric enzyme complex, nitrogenase. The nitrogenase complex exists in all diazotrophs. It consists of two conserved proteins: an iron (Fe)-containing dinitrogenase reductase (Fe protein) encoded by the *nifH* gene and a molybdenum iron (Mo Fe) dinitrogenase (or Mo Fe protein), which is encoded by the *nifDK* genes (Matthew et al., 2008). The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin), HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism, reduced ferredoxin, which supplies electrons for this process, is generated by photosynthesis, respiration or fermentation. There is a remarkable degree of functional conservation between the nitrogenase proteins of all nitrogen-fixing bacteria. The Fe protein and the Mo-Fe protein have been isolated from many of these bacteria, and nitrogen fixation can be shown to occur in cell-free systems in the laboratory when the Fe protein of one species is mixed with the Mo-Fe protein of another bacterium, even if the species are very distantly related. The nitrogenase is irreversibly inhibited by molecular oxygen and reactive oxygen species, because the oxygen reacts with the iron component of the proteins. Although this is not a problem for anaerobic bacteria, it could be a major problem for the aerobic species such as cyanobacteria (which generate oxygen during photosynthesis) and the free-living aerobic bacteria of soils, such as *Azotobacter* and *Beijerinckia*. These microorganisms have various defense mechanisms to overcome the problem. For example, *Azotobacter* species have the highest known rate of respiratory metabolism of any organism, so they might protect the enzyme by maintaining a very low level of oxygen in their cells. These species also produce extracellular polysaccharide, which retains water and in this way limits the diffusion rate of oxygen to the cells.

Plant growth-promoting bacteria (PGPB) have been used as biofertilizers worldwide, due to their ability to promote plant growth and therefore crop yields and soil fertility and hence, the potential to contribute to more sustainable agriculture and forestry.

Generally, PGPB facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development, in the form of biocontrol agents. They suppress the activity of pathogens by producing numerous metabolites like siderophores, hydrolytic enzymes, and antibiotics. PGPB live freely in soil, colonize plant roots aggressively and establish symbiotic association with plants. The existence of PGPB with the plant roots is generally classified by two environments;

rhizosphere and endosphere. The rhizosphere is the soil volume under the direct influence of roots, while the endosphere is the internal root tissue. The strains inhabiting the rhizosphere and endosphere are called rhizobacteria and endophytes, respectively.

Only N-fixing microorganisms bring additional supplies of a nutrient (N) into the soil/plant system. All other biofertilizers simply solubilize or mobilize the nutrients that are already present in soils. Microorganisms that have the capacity to fix atmospheric N_2 can be used as efficient biofertilizers. Their application in soil improves the soil biota and reduces the need of chemical fertilizers. Among all PGPB, the diazotrophic (N_2-fixing) bacteria, which are involved in the transformation or fixation of N_2 from the unavailable gaseous form in the atmosphere, are divided into:

- Free-living heterotrophic or autotrophic bacteria;
- Bacteria in associative symbiotic relationships;
- Bacteria in symbiotic relationships with plants.

Free-living nitrogen fixers

The free-living, or non-symbiotic, nitrogen-fixing bacteria live outside plant cells and are associated with the rhizosphere, the part of soil under the influence of plant roots and their exudates. They are of four types:

- Free-living non-photosynthetic aerobic nitrogen-fixing bacteria such as *Azotobacter*, *Beijerinckia* and *Derxia*;
- Free-living non-photosynthetic anaerobic nitrogen-fixing bacteria such as *Clostridium*;
- Free-living photosynthetic nitrogen-fixing bacteria such as *Chromatium*, *Rhodopseudomonas*, *Rhodospirillum*, cyanobacteria;
- Free-living chemosynthetic nitrogen-fixing bacteria such as *Desulfovibrio*.

Free-living non-photosynthetic nitrogen-fixing bacteria

Although many genera and species of N_2-fixing bacteria are isolated from the rhizosphere of various cereals, mainly members of the *Azotobacter* and *Azospirillum* genera have been widely tested to increase the yield of cereals and legumes under field conditions. *Azotobacter* is an obligate aerobe, although it can grow under limited O_2 concentration. Its six species are: *Azotobacter armeniacus*, *A. beijerinckii*, *A. chroococcum*, *A. nigricans*, *A. paspali* and *A. vinelandi*. These species play an important role in nitrogen fixation in rice crops and are used as a biofertilizer for wheat, barley, oat, rice, sunflower, maize, line, beetroot, tobacco, tea, coffee and coconuts. *Azotobacter* species are different in terms of morphological and physiological characteristics. Some of them have higher nitrogen-fixing ability than others. Inoculation of soil with *Azotobacter* species lead to increase in crop yields due to the increase in the concentration not only of nitrogen, but also of other substances, such as vitamins, gibberellins, naphthalene and acetic acid, which improve plant growth. *Azotobacter* also synthesizes growth-promoting substances, produces group B vitamins such as nicotinic acid and pantothenic acid, biotin and heteroauxins, gibberellins and cytokinin-like substances, and improves the seed germination in several crops. Both carrier-based and liquid-based *Azotobacter* biofertilizers are available.

Free-living photosynthetic nitrogen-fixing bacteria

Free-living nitrogen-fixing photosynthetic cyanobacteria (blue-green algae) belong to 15 genera, which are found freely in the soil where they fix free N_2 into nitrogenous and ammonium compounds. Mostly they are heterocysts, e.g *Nostoc*, *Anabaena*, *Aulosira*, *Cylindrospernum*, *Calothrix, Totypothrix* and *Stigonema*. Cyanobacteria are photosynthetic and hence add organic matter and extra nitrogen into the soil. Amongst these, *Aulosira* is the most active nitrogen fixer in the rice fields of India. Nitrogen fixation occurs in special thick walled cells called heterocysts, or heterocytes (H), which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO_2 to produce organic products) and oxygen. There are fewer non-heterocystous nitrogen-fixing blue-green algae, e.g. *Oscillatoria, Phormidium* and *Gleocapsa*.

Associative symbiotic nitrogen fixers

This group comprises bacteria from the family Spirillaceae with two main genera, *Azospirillum* and *Herbaspirillum*. Bacteria of the genus *Azospirillum* are widespread in the soils of tropical, subtropical and temperate regions where they live in symbiotic mutualism around the root of various wild and agricultural plants, which is also known as a risosphere association. They are a good example of the so-called associative nitrogen fixers. *Azospirillum* belong to the facultative endophytic diazotrophs groups, which colonize the surface and the interior of non-legume plants. They are able to fix a considerable quantity of nitrogen in the range of 20– 40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton, rice, sugar cane etc. Nitrogen fixers such as *Azospirillum* benefits plant by improving shoot and root development and increasing the rate of water and mineral uptake by roots (Gonzales et al., 2005). The yield increases can be substantial, up to 30 percent, but generally range from 5 to 30 percent. These yield increases by *Azospirillum* are possibly a result of the production of growth-promoting substances rather than N_2 fixation (Okon, 1985). The main problem that limits the use of *Azospirillum* on a large scale is the great uncertainty and unpredictability of the results. Regardless of these uncertainties, *Azospirillum* bears great promise as a growth-promoting N_2-fixing biofertilizer. The species *A. lipoferum*, *A. brasilense* and *A. amazonense* have been commercially used as nitrogen-supplying biofertilizers.

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Symbiotic nitrogen fixers

The best known and most exploited symbiotic nitrogen fixers comprise mutualistic (symbiotic) bacteria belonging to the group of Alphaproteobacteria, family Rhizobiaceae, which include the following genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium* and *Allorhizobium*, collectively called rhizobia. Rhizobia participate in mutually useful associations with the roots of leguminous plants where they form noodles and carry out the nitrogen fixation process. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g. alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium. *Rhizobium* can fix 15–20 kg N/ha and increase crop yields up to 20% in pulses. It has been estimated that 40–250 kg N/ha/year is fixed by different legume crops by the microbial activities of *Rhizobium*. The N_2-fixing capability of rhizobia varies significantly among host plant species and bacterial strains.

Therefore, for the production of biofertilizers not only the bacterial strain, but also the rhizobia-host compatibility must be taken into account.

The N_2-fixers from the genus *Frankia* also participate in symbiotic relationships with certain dicotyledonous species (actinorhizal plants). *Frankia* are a free-living gram-positive filamentous actinobacteria found in root nodules or soil. Inoculation of actinorhizal plants with *Frankia* significantly improves plant growth, biomass, shoot and root N content, as well as the survival rate after transplanting in fields. However, the success of establishment of an actinorhizal plantation in degraded sites depends upon the choice of effective *Frankia* strains. Species from this genus are capable of infecting and nodulating eight families of actinorhizal plants (mainly woody plants), which are used for wood production, land reclamation, for timber and fuel wood production, in mixed plantations, for windbreaks, as well as for shelterbelts along deserts and coastlines. *Frankia* inoculation can be advantageous in arid environments, disturbed sites, and areas where native actinorhizal plants are absent. The symbiosis between actinorhizal plants and *Frankia* induces the formation of a perennial root organ called nodule, wherein bacteria are hosted and nitrogen is fixed. In the field, actinorhizal nodules can have variable forms and colours. Comparison of actinorhizal and leguminous nodules shows that the morphology, anatomy, origin, and functioning of the nodules are different for these two nitrogen-fixing plants. Two types of nodule formation occur in actinorhizal symbiosis: intercellular and extracellular infection.

Cyanobacteria are ecologically important because they contribute significantly to the global N_2-fixation. Their capability to fix molecular nitrogen is essential in rice cultivation and in the remediation of arid soils. Nevertheless, the production and application of cyanobacteria is still fairly poorly developed. However, cyanobacteria should be seriously considered as a biofertilizer supporting sustainable agricultural practices in various environments.

Besides cyanobacteria (blue-green algae), which are an important biological factor in rice cultivation, *Azolla* forms another inexpensive, economical, and ecologically friendly biofertilizer. The important factor in using *Azolla* as biofertilizer for rice crops is its quick decomposition in the soil, efficient availability of its nitrogen to rice plants, requirement of a shallow freshwater habitat, rapid growth, and growth along with rice without competition for light and space. Increase in grain yields of rice from 14% to40 % have been reported with *Azolla* being used as a dual crop. It improves the height of rice plants, the number of tillers, grains and the straw yield. It is supplemented with 8–20 kg phosphate per hectare.

Besides N-fixation, these biofertilizers or biomanures also contribute significant amounts of P, K, S, Zn, Fe, Mb and other micronutrients. Widely cultivated in the Asian regions, *Azolla* is either incorporated into the soil before rice transplanting or is grown as a dual crop along with rice. The Asians have recognized the benefits of growing *Azolla* as a biofertilizer, human food and medicine. It also improves water quality by removal of excess quantities of nitrate and phosphorous and is also used as fodder, feed for fish, ducks and rabbits. *Azolla* is a small floating pteridophyte which has symbiotic associations with cyanobacteria and eubacteria that remain associated throughout its life cycle. It is unique in the sense that it acts as a host to the N-fixing cyanobacteria, after which it is used virtually as a green manure. In this process, it adds not only the biologically fixed N, but also the other nutrients absorbed from the soil and present in its biomass. There are seven species of the Azzolaceae family: *Azolla caroliniana*, *A. filiculoides, A. maxicana*, *A. microphylla*, *A. pinnata*, *A. rubra* and *A. nilotica*. In India, *A. pinnata* is commonly observed. The algal symbiont belongs to family Nostocaceae and is generally referred to as *Anabaena azollae*. In the associations between *Azolla* and the cyanobacteria *Anabaena azollae*, the eukaryotic partner *Azolla* houses the prokaryotic endosymbiont *Anabaena azollae* in its leaf cavities and provides carbon sources and, in turn, gets its nitrogen requirements satisfied. The atmospheric nitrogen is harvested by the algal symbiont. The sites of nitrogen fixation are heterocysts. The heterocyst counts increase along the stem from the apex towards the base in the successive leaves. This symbiosis helps in the quick growth and multiplication of the fern and in the creation of a huge amount of biomass on the water surface. It is then harvested, dried and used as biofertilizer to supplement the needs of nitrogen in coffee farms as well.

Phosphorus biofertilizers

Phosphorous (P) is the next essential macroelement after nitrogen. Phosphorus is required in a soluble form for maximizing crop growth and production. It plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate the metabolic pathways. The phosphate available in soil occurs in three forms: soil solution phosphate, insoluble organic phosphate and insoluble inorganic phosphate. The greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates. This means that soils contain a high amount of total phosphorus, but its availability to plants is very low and it is often a limiting factor for plant growth.

A major characteristic of phosphorus biogeochemistry is that only 1% of the total soil phosphorus (400–4,000 kg P/ha in the top 30 cm) is incorporated into living plant biomass during each growing season (10–30 kg P/ha), reflecting its low availability for plant uptake. Phosphorus deficiency in plants leads to chlorosis, weak stem and slow growth. Therefore, it is considered to be the most important chemical factor that restricts plant growth because of its vital role in the physiological and biochemical functions of plants. The application of chemical phosphorous fertilizers to circumvent the phosphorus deficiency in soil is not a very efficient method due to the high reactivity of phosphate anions through precipitation with cations such as Fe^{3+} and Al^{3+} in acidic soils or Ca^{2+} in calcareous soils. The application of microbial inoculants with phosphate-solubilizing activity will be a promising approach to increase the phosphorus availability in agricultural soil and is an environmentally-friendly alternative to the use of chemical fertilizers. Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus-containing compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which release orthophosphate from the carbon structure of molecules. Various bacterial species are able to solubilize inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphates. It is important to determine the actual mechanism of phosphorus solubilisation by PSM for optimal utilization of these microorganisms in various field conditions. Microorganisms must assimilate phosphorus via membrane transport, so dissolution of calcium phosphate [$Ca(H_2PO_4)_2$] to dihydrogen phosphate anion ($H_2PO_4^-$) is considered essential to the global phosphorus cycle.

The solubilization of phosphorus in nature is due to the activity of phosphate-solubilizing microorganisms (PSM) which belong to several genera: *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia.* The symbiotic nitrogenous rhizobia, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, also show phosphate-solubilizing activity. For instance, *Rhizobium leguminosarum* bv. *trifolii*, and *Rhizobium* species nodulating *Crotalaria* species improved plant phosphorus nutrition by mobilizing inorganic and organic phosphorus. Various phosphate-solubilizing bacteria have also been isolated from stressed environments; for example, the halophilic bacteria *Kushneria sinocarni* isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in salt-affected agricultural soils.
Two types of phosphate biofertilizers have been developed based on the application of phosphate-solubilizing bacteria and phosphate-mobilizing microorganisms.

Phosphate-solubilizing biofertilizers

The members of this group are bacterial and fungal species which solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate. The most efficient ones belong to *Bacillus* and *Pseudomonas* among Bacteria and *Aspergillus* and *Penicillium* among Fungi. They could be isolated in higher concentrations from rhizosphere soil rather than non-rhizosphere soil. Their application in biofertilizers aims to increase the yields of legume, cereals, vegetables and fruit crops. The phosphate-solubilizing fungi produce more acids than bacteria and consequently exhibit greater phosphate-solubilizing activity. Among the filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* are the most representative ones, although strains of *Trichoderma* and

Rhizoctonia solani have also been reported as phosphate solubilizers. A number of theories have been proposed to explain the mechanisms of phosphate solubilization. The most important theories are the acid production theory and the proton and enzyme theory.

- *Acid production theory*

The major mechanism involved in the solubilization of phosphate by phosphate-solubilizing microorganisms is the production of organic acids which either directly dissolve rock phosphate as a result of anion exchange of phosphate by acid anion or chelate Fe, Al, Ca ions to bring the phosphate into solution. Due to the ability of PSM to secrete and release organic acids (citric, oxalic, succinic, tartaric, malic, alpha keto butyric, 2-ketogluconic, gluconic and fumaric acids) in the soil environments, these bacteria lower the pH in their vicinity, which is a prerequisite for solubilization of bound phosphates in soil and consequently dissociate the bound form of phosphates like $Ca_3(PO_4)_2$ in calcareous soil. The microbial organic acids are produced as a result of oxidative respiration or by fermentation of organic carbon sources. Gluconic and fumaric acids have the highest ability to solubilize phosphate from inorganic phosphate compounds. The amount of soluble phosphate released depends on the strength and type of acid. Aliphatic acids are found to be more effective in phosphate solubilization than phenolic acids and citric acids. *Pseudomonas* sp., *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia* are phosphorus-solubilizing bacteria, which produce a higher amount of gluconic acid. Besides organic acids, inorganic acids such as nitric and sulphuric acids are also produced by the nitrifying *Nitrosomonas* and sulphur-oxidizing *Thiobacillus* bacteria during the oxidation of nitrogenous or inorganic compounds of sulphur which react with calcium phosphate and convert them into soluble forms. The introduction of efficient phosphate solubilizers in the rhizosphere of crops increases the availability of phosphorus and thus increases the crop yield up to 200–500 kg/ha. In this way, microorganisms play a major role in the solubilization and uptake of native and applied phosphorus.

- *Enzyme and proton theory*

Phosphate-solubilizing microorganisms are also known to produce phosphatase enzyme along with acids which cause the solubilization of phosphate in aquatic environment. Esterases are involved in liberating phosphorous from organic compounds. Solubilization without acid production is due to the release of protons accompanying respiration or ammonium assimilation. Besides these mechanisms, some bacterial species synthesize syderophores – iron-chelating compounds which bind the iron present in the root area and, thus, make it unavailable for harmful microorganisms so that crop plants are protected from them. The production of other chelating substances, mineral acids and biologically active substances like indole, acetic acids, gibberellins and cytokinins, is also correlated with phosphate solubilization.

Phosphorus mobilizing biofertilizers: Mycorrhiza

This type of biofertilizers contain mycorrhizal fungi also known as phosphate absorbers. They are a heterogeneous taxonomic group which inhabits the plant root system and establishes a symbiotic association with them. Mycorrhizal fungi live in symbiosis with over 90 % of all vascular plant species, including many important crop species, such as maize, wheat, rice and potato. Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots. There are two major types of mycorrhizae: ectomycorrhizal fungi (EM) and endomycorrhizal fungi (AM). Endomycorrhizae are the most common type, and are found in grasses, shrubs, some trees and many other plants. Ectomycorrhizal fungi are usually specific to a certain host species, but most species of endomycorrhizae will form relationships with almost any AM-fungi host plant, and are therefore much easier to specify. The arbuscule-forming mycorrhiza (AMF) are a widespread type of endomycorrhiza associated with crop and horticultural plants, where fungal hyphae of *Glomeromycota* species penetrate root cortical cells and form branched structures called arbuscules. The host plant is benefited by obtaining needed nutrients, especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. Phosphorus is a highly immobile element because it is easily absorbed by soil particles and a phosphate-free zone rapidly occurs around plant roots. Some of the external hyphae of mycorrhizal fungi may extend more than 10 cm from the root surface, which allows them to have access to a greater volume of non-depleted soil than the root alone. The small diameter of hyphae (20 to 50 µm) permits access to soil pores that cannot be explored by roots as well. They also produce extracellular alkaline phosphatases which can mobilize phophate from organic sources. Through the excretion of protons, hydroxyls and organic acids, mycorrhizae modify the redox potential around the root and the mycelium, which also enhances the transformation of insoluble phosphate from the soil into a soluble form in the soil solution. Therefore, a root system forming a mycorrhizal network will have a greater effective surface area for absorbing nutrients and exploring a greater volume of soil than nonmycorrhizal roots. AM hyphae also excrete gluey, sugar-based compounds called glomalin, which helps to bind soil particles, and make stable soil aggregates. There is an increasing interest in the use of mycorrhiza to promote sustainable agriculture, considering the widely accepted benefits of the symbioses to nutrition efficiency (for both macronutrients, especially P, and micronutrients), water balance and biotic and abiotic stress protection of plants. Vesicular Arbuscular Mycorrhiza Root Inoculant (VAMRI) is a biofertilizer based on chopped dried corn roots infected with *Glomus* species (*G. mosseae* or *G. fasciculatum*). Besides a microbial inoculant, this product also serves as a biocontrol agent of soil-borne diseases of different crops under various conditions. VAMRI can be applied for pepper, tomato, papaya, onion, corn, peanut, sugarcane, eggplant, banana, fruit crops, watermelon, etc.

Potassium (K)-solubilizing biofertilizers

Potassium (K) is the third essential nutrient necessary for plant growth. Some rhizobacteria are able to solubilize insoluble potassium forms. *Bacillus edaphicus* has been reported to increase potassium uptake in wheat and *Paenibacillus glucanolyticus* has been found to increase the dry weight of black pepper. Sudan grass inoculated with the potassium-solubilizing bacterium *Bacillus mucilaginosus* had higher biomass yields. Moreover, *Bacillus mucilaginosus* in co-inoculation with the phosphate-solubilizing *Bacillus megaterium* promoted the growth of eggplant, pepper and cucumber.

Biofertilizers for secondary macronutrients: zinc and iron solubilizers

Zinc is of utmost importance. It is found in the earth's crust at a concentration of 0.008%, but there are soils which exhibit zinc deficiency with content far below the critical level of 1.5 ppm of available zinc. The plant deficiencies in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate ($ZnSO_4$). Microorganisms found in the soil can be used as biofertilizers to provide micronutrients like Zn, Fe, Cu, etc. Zinc can be solubilized by *B. subtilis*, *Thiobacillus thioxidans* and *Saccharomyces* sp. These species are responsible for Zn extraction in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate. The zinc fixation occurs through two main mechanisms: the first one operates in acidic soils and is based on cation exchange; the second mechanism operates in alkaline soils where fixation takes place by sorption of Zn on $CaCO_3$ and, as a result, a solid-solution of $Zn_{\chi}Ca_{\chi-1}CO_3$ is formed.

Plant-growth-promoting rhizobacteria (PGPR)

A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant-growth-promoting rhizobacteria or PGPR. PGPR is a generic acronym that indicates bacteria which, in some often unknown way, can stimulate plant growth. They belong to several genera, e.g. *Agrobacterium*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorphosporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas*. These bacteria vary in their mechanism of plant growth promotion but generally influence growth via phosphate solubilization, nutrient uptake enhancement, plant growth hormone production or production of a variety of antimicrobial compounds that act in different ways. Bertrand et al. (2000) showed that a rhizobacterium belonging to the genus *Achromobacter* could enhance the root hair number and length in oilseed rape (*Brassica napus*). *Achromobacter* increased the NO_3 and K uptake and, consequently, the shoot and root dry weights by 22 to 33 percent and 6 to 21 percent, respectively. One of the plant-growth-promoting mechanisms of rhizobacteria is the antagonism against phytopathogenic microorganisms due to the production of antimicrobial metabolites like siderophores, antibiotics, cyanides, fungal cell-wall-degrading enzymes and gaseous products including ammonia (Idris et al., 2007; Lugtenberg and Kamilova, 2009). The mechanism of antifungal effects lies in the production of a variety of antimicrobial compounds that act in different ways. The antagonistic effects are caused by cytolysis, leakage of potassium ions, disruption of the structural integrity of membranes, inhibition of mycelial growth and protein biosynthesis. Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites such phenazines, pyrrolnitrin, pyoluteorin and cyclic lipopeptides like viscosinamide. It was demonstrated that viscosinamide prevents the infection of sugar beet by *Pythium ultimum*. These bacterial strains, besides having an antagonistic effect, also influence the defense system of plants. The siderophore-mediated competition for iron is one of the mechanisms responsible for the antagonistic activity of *Pseudomonas* spp. The secreted iron-chelating compounds bind ferric ions (Fe^{3+}), and are taken up by microbial cells through specific recognition by membrane proteins (Srivastava and Shalini, 2008). The presence of iron-chelating compounds makes the bacteria better competitors for iron, in this way preventing the growth of pathogenic microorganisms. *Pseudomonas* species produce two different types of siderophores: pseudobactin and pyoverdin (Oldal et al., 2002). Siderophores produced by biocontrol bacteria have a higher affinity for iron than those produced by some fungal pathogens, allowing the former microbes to scavenge most of the available iron, preventing the proliferation of fungal pathogens (Hillel, 2005). Some authors have reported that *Pseudomonas fluorescens* belonging to the PGPR class produces siderophores and has a biocontrol effect against *P. ultimum*, *R. batatticola* and *Fusarium oxysporum*. Other *Pseudomonas* species like *Pythium stutzeri* produce extracellular enzymes like chitinase and laminase capable of lysing the mycelia of *Fusarium solani*. *Pseudomonas aeruginosa* produces three types of siderophores under iron-limiting conditions: pyoverdine, pyochelin and its precursor salicylic acid, and induces resistance to plant diseases caused by *Botrytis cinerea* on bean and tomato, *Colletotrichum lindemuthianum* on bean. *F. oxysporum* causes vascular wilt and foot-, root- and bulbrot diseases in a wide variety of economically important crops. *Alternaria* spp., *Sclerotium* spp. cause leaf spots, root rot and stem rot, which also leads to serious yield losses. The antifungal effect of PGPRs is influenced by a lot of environmental and genetic factors. Biotic and abiotic environmental signals may have an important input on the regulation of biocontrol genes in pseudomonads, e.g. on the repression of siderophore biosynthesis. Together with low oxygen concentrations, the available carbon and nitrogen sources that influence the molecular mechanisms are involved in biocontrol activity.

Compost as fertilizer

What is compost?

Composting is a controlled microbial bio-oxidative process in which organic biodegradable wastes are converted into a hygienic, humus-rich product (compost) for use as a soil conditioner and an organic fertilizer. It is an inexpensive, efficient, and sustainable treatment for solid wastes. The process is dependent on a number of factors, including temperature, moisture (typically 40–60% by weight), sufficient oxygen to support an aerobic environment (typically 5% or more), particle size, the C/N ratio and the degree of turning involved. The effective management of these factors will accelerate the composting process. Compost can be defined as organic manure or fertilizer produced as a result of aerobic, anaerobic or partially aerobic decomposition of a wide variety of crop, animal, human and industrial wastes. Composting has a long tradition almost everywhere in the world. It was a central concept of early Chinese agriculture, but it has also been practiced in India and Europe for centuries. Compost is a dark, crumbly, earthy material, which usually contains less than 2% (*w*/*w*) of nitrogen, phosphorous and potassium (N:P:K). It also has microscopic fungi, bacteria, earthworms and dung beetles. This mixture creates a symbiotic food web within the soil. The decomposing material feeds the organisms and helps to aerate the soil while also keeping it moist. The nutrient value of composts varies widely, depending upon the nature of feedstock composted.

Composts are generally classified as:

- Rural compost: This is produced from materials available on the farm and in other rural areas. The raw materials used can be straw, leaves, cattle-shed bedding, fruit and vegetable wastes, and biogas plant slurry. On average, it contains 0.5% N, 0.2% P_2O_5 and 0.5% K_2O. Rural compost primarily finds use on farms as bulky organic manure.
- Urban or town compost: This refers to compost prepared from urban and industrial wastes, city garbage, sewage sludge, factory waste, etc. Its typical composition is 1.5–2.0% N, 1.0% P_2O_5 and 1.5% K_2O. Commercially prepared urban compost has been reported to contain 1% Fe, about 375 mg/kg Cu, 705 mg/kg Zn, 740 mg/kg Mn and small amounts of other micronutrients.
- Vermicompost: This is an important type of compost that contains earthworm cocoons, excreta, beneficial microorganisms, actinomycetes, plant nutrients, organic matter, enzymes, hormones, etc. It is an organic fertilizer produced by earthworms and contains on average 0.6% N, 1.5% P_2O_5 and 0.4% K_2O. In addition to NPK, it is also a source of micronutrients, containing an average of 22 mg/kg Fe, 13 mg/kg Zn, 19 mg/kg Mn and 6 mg/kg Cu. It helps in cost-effective and efficient recycling of animal wastes (poultry, horse, piggery excreta and cattle dung), agricultural residues and industrial wastes using low energy.

Various parameters are commonly used to evaluate compost quality. In general, these parameters include germination index (GI), water-soluble organic carbon (WSOC), water-soluble organic nitrogen (WSON), pH, electrical conductivity (EC), moisture and total organic matter (TOM) content. It is accepted that any sole parameter cannot determine the compost maturity, which must be assessed by a combination of different physical (odour, colour, temperature and particle size), chemical (C/N ratio, mineral N, pollutants content (heavy metals and organics), pH, organic matter quality and humification) and biological properties (microbial activity indicators such as respiration, ATP content, enzyme activity, microbial biomass, nitrogen mineralization). The pH of the mature compost is usually around 7.5 and it has a C:N ratio ranging from 10:1 to 20:1. The temperature in the pile is equal to that of the surrounding air. Compost smells earthy, no longer heats up after turned or watered, looks like dark soil, and does not have identifiable food items, leaves or grass. The application of immature compost to soil results in seed germination inhibition, root destruction, and a decrease in the O_2 concentration and redox potential, which imposes the need to assess the compost maturity.

Compost benefits and use

When applied to soil, composts (organic manure) or compost extracts have beneficial effects on plant growth and are considered as a valuable soil amendment. Compost application is very popular as a means of improving the soil physical properties and supplying plant nutrition. It also provides nutrients rich in organic carbon for the microbial biomass, which converts the unavailable nutrients in plant residues to ones available for crops, and it enhances the biodiversity of soil microorganisms. Organic fertilizers (animal/plant based) also activate the natural microflora in the soil and rhizosphere of the plant and are excellent means of enhancing the natural microbial population. Composts contain macro and micronutrients that are often absent in synthetic fertilizers and release nutrients slowly—over months or years, unlike synthetic fertilizers. Composts buffer the soil, neutralizing both acid and alkaline soils, bringing the pH levels to the optimum range for nutrient availability to plants. Composts help bind clusters of soil particles, called aggregates, which provide good soil structure. Such soil is full of tiny air channels and pores that hold air, moisture and nutrients. This makes any soil easier to work and is also useful for erosion control. Erosion is often the end result of low soil fertility. Compost and the humus it contains can actually bind to soil, building a good structure that encourages optimum fertility and erosion resistance. A comparatively new application for compost is bioremediation. Many things can contaminate surface waters, soils and reservoirs. The microorganisms in compost degrade contaminants in water or soil. Contaminants are digested, metabolized and transformed into humus and inert byproducts such as carbon dioxide, water and salts. Compost bioremediation is effective in degrading or altering chlorinated and non-chlorinated hydrocarbons, wood-preserving chemicals, solvents, heavy metals, pesticides, petroleum products and explosives.

Microbial community in compost

During composting, different animal/plant wastes like dead plants, farm yard waste and cattle waste are degraded by various decomposing microorganisms with cellulolytic/ lignolytic activity such as *Trichoderma viridae*, *Aspergillus niger*, *Aspergillus terreus*, *Bacillus* sp., etc. Composts support high population levels of bacteria with higher percent of Gram-negative cultures. Some isolates show proteolytic activity, which is considered a potential mechanism of suppression or competition with other microorganisms. The major Gram-negative genera identified in mature compost are *Pseudomonas*, *Serratia*, *Klebsiella*, and *Enterobacter*. All Gram-positives are identified as *Bacillus* spp. The essential elements required by the composting microorganisms are carbon, nitrogen and oxygen, as well as moisture. If there is a lack of any of these elements, or if they are not provided in the proper proportion, the microorganisms will not flourish and will not provide adequate heat. A composting process that operates at optimum performance will convert organic matter into stable compost that is odour and pathogen free, and a poor breeding substrate for flies and other insects. In addition, it will significantly reduce the volume and weight of organic waste, as the composting process converts much of the biodegradable component to gaseous carbon dioxide.

The composting period is governed by a number of factors including, temperature, moisture, oxygen, particle size, the carbon-to-nitrogen ratio and the degree of turning involved. Generally, effective management of these factors will accelerate the composting process.

Compost preparation

The composting process is carried out by three classes of microbes:

- Psychrophiles - low temperature microbes;

- Mesophiles -medium temperature microbes;
- Thermophiles - high temperature microbes.

Generally, composting begins at mesophilic temperatures and progresses into the thermophilic range. This is due to the oxidative metabolism of microorganisms, which is exothermic and the heat produced is sufficient to increase the temperature of organic matter to 65–75 °C over a period of up to 10 days. The thermophilic stage of composting appears as a self-sanitizing mechanism by which pathogens, thermolabile microbial and plant toxins are destroyed. Temperature is directly proportional to the biological activity within the composting system. As the metabolic rate of the microbes accelerates, the temperature within the system increases. Conversely, as the metabolic rate of the microbes decreases, the system temperature decreases. Not all organic matter is degraded completely. Lignin, lignocellulosic and other plant components are modified slowly and become part of the final stable compost. Soluble plant exudates and sap are bio-degraded more rapidly. After the most readily decomposable organic matter in the compost is consumed, the biological activity decreases in intensity, and the temperatures and oxygen consumption decline. The compost then enters the curing phase, during which decomposition proceeds more slowly and organic matter is converted to stable humic substances—the finished or mature compost. Crops residues are compostable matter but, although high in carbon, they are deficient in nitrogen. On the contrary, animal wastes are rich in nitrogen and very often low in carbon content.

Compost as a plant protectant

Compost can be transformed into suppressive compost after inoculation of biological control agents specifically active against a plant disease. In practice, composts are not consistently or naturally colonized by a broad spectrum of biocontrol agents because the latter are destroyed by high temperatures during active composting. To be effective, biocontrol agents must recolonize composts during the curing process and this does not always occur. For example, composts produced near a forest are much more likely to become colonized by effective biocontrol agents and more consistent in suppressing rhizoctonia diseases than those produced in an enclosed system. Microbes that show a preference for colonizing and lysing plant pathogens might be classified as biocontrol agents.

The microorganisms stimulated by compost amendments contribute to the suppressive activity of the amended soil through four control mechanisms: antibiosis, competition, parasitism and induced systemic resistance.

Antibiosis is the inhibition of one organism's growth by a metabolic product such as antibiotic produced by another organism. Agrobacterium radiobacter 84 produces bacteriocin, called agrocin, which is a widely accepted commercial product for controlling of crown gall – a serious disease of stone-fruit trees in nurseries and of many other woody plants. Lysobacter and Myxobacteria are known to produce copious amounts of lytic enzymes, and some isolates have been shown to be effective at suppressing fungal plant pathogens. Expression and secretion of these enzymes by different microbes can sometimes directly result in the suppression of plant pathogen activities. For example, control of Sclerotium rolfsii by Serratia marcescens appeared to be mediated by chitinase expression. Some products of lytic enzyme activity may contribute to indirect disease suppression. For example, oligosaccharides derived from fungal cell walls are known to be potent inducers of plant host defenses. The enzyme β-1,3-glucanase contributes significantly to biocontrol activities of Lysobacter enzymogenes strain C3.

Competition is when microorganisms compete for nutrients such as high-energy carbohydrates, nitrogen and iron, as well as for infection sites, oxygen and space.

An example of parasitism are parasitic fungi which invade plant pathogens resulting in lysis and death. Effective control of Rhizoctonia solani can be achieved by applying isolates of Trichoderma species combined with any of several bacterial biocontrol agents. The representatives of the Trichoderma genera are the main microorganism isolated from compost prepared from lignocellulosic wastes and capable of parasitizing Rhizoctonia solani.

The mechanism of induced systemic resistance is based on activation of the production of plant metabolites such as salicylic acid, defense-related proteins or other compounds which lead to systemic plant resistance to pathogens. Some biocontrol strains of Pseudomonas sp. and Trichoderma sp. are known to strongly induce plant host defenses. In several instances, inoculations with plant-growth-promoting rhizobacteria (PGPR) were effective in controlling multiple diseases caused by different pathogens, including anthracnose (Colletotrichum lagenarium), angular leaf spot (Pseudomonas syringae pv. lachrymans and bacterial wilt (Erwinia tracheiphila).

The quantitative contribution of biologically active compounds to disease suppression is likely to be dependent on the composition and carbon-to-nitrogen ratio of the soil organic matter that serves as a food source for microbial populations in the soil and rhizosphere. However, such activities can be manipulated so as to result in greater disease suppression. When suitable antagonists are already presented in the soil or substrate but do not provide a satisfactory level of disease control, their activity must be intensified. For example, in post-harvest disease control, addition of chitosan can stimulate microbial degradation of pathogens similar to that of an applied hyperparasite. Chitosan is a non-toxic and biodegradable polymer of beta-1,4-glucosamine produced from chitin by alkaline deacylation. Amendment of the plant growth substratum with chitosan suppressed root rot caused by Fusarium oxysporum f. sp. radicis-lycopersici in tomato. Although the exact mechanism of action of chitosan is not fully understood, it has been observed that treatment with chitosan increases the resistance to pathogens. The extent to which composts suppress this disease depends on the chemical-physical nature of the composted materials and increases with the compost maturity.

COMMON-USED BIOFERTILIZERS

TYPES OF BIOFERTILIZERS ON THE BASIS OF THE PHYSICAL NATURE AND CARRIER MATERIALS USED

Based on the physical nature and carrier materials used, various types of biofertilizers are manufactured by different producers. These are carrier-based inoculants, agar-based inoculants, broth cultures and dried cultures. New developments in biofertilizer production like (i) freeze-dried inoculants (e.g. BAIF, IARI, India), (ii) Rhizobium-paste (e.g. KALO Inc. USA), (iii) granular inoculant (e.g. Soil implant of Nitragin, USA), (iv) pelleting (e.g. Pelinoc of Nitragin), (v) polyacrylamide-entrapped rhizobia (e.g. Agrosoke) and (vi) pre-coated seeds (e.g. Prillcote of New Zealand), appear to be more promising for inoculation success in tropical legumes.

Carrier-based biofertilizers

At present, biofertilizers are supplied as carrier-based microbial inoculants which are added to the soil to enrich the soil fertility. The carrier is a medium that can carry the microorganisms in sufficient quantities and keep them viable under specified conditions, easy to supply to the farmers. The use of ideal carrier material is necessary in the production of good quality biofertilizer.

A good carrier should have the following qualities:

- Highly absorptive (water-holding capacity) and easy to process;
- Non-toxic to microorganisms;
- Easy to sterilize effectively;
- Available in adequate amounts and low-cost;
- Provide good adhesion to seeds;
- Has good buffering capacity;
- High organic matter content and water-holding capacity of more than 50%.

Other essential criteria for carrier selection relating to the survival of the inoculant bacteria should be considered.

- Survival of the inoculant bacteria on seeds. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into soil.
- Survival of the inoculant bacteria during the storage period.
- Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche, and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micro-pores to the inoculant bacteria will be desirable. In this sense, materials with micro-porous structure, such as soil aggregate and charcoal, will be good carriers for soil inoculants.

COMMON-USED BIOFERTILIZERS

Biofertilizers are supplied to the soil either by “seed inoculation”, in which the inoculant (bacteria-carrier mixture) is mixed with water to make slurry-form and then mixed with seeds, or by “soil inoculation”, i.e. by spreading over the field during cultivation. In the case of seed inoculation, the carrier must be a form of fine powder. To achieve a tight coating of inoculant on the seed surface, use of an adhesive, such as gum arabic, methylethylcellulose, sucrose solutions and vegetable oils, is recommended. Seed inoculations may not always be successful due to the low nodule occupancy of the inoculated rhizobia strain as a result of the inoculation or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, “soil inoculation” will be adopted, whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots. Various types of material are used as carriers for seed or soil inoculation. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. Neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production. For preparation of seed inoculant, the carrier material is milled to fine powder with a particle size of 10–40 µm. For soil inoculation, carrier material with granular form (0.5–1.5 mm) is generally used. Granular forms of peat, perlite, charcoal or soil aggregates are suitable for soil inoculation.

Liquid biofertilizers

The strength of biofertilizers is determined by two basic parameters: number of cells and efficiency of the microorganisms to fix nitrogen or solubilize phosphates.

Liquid biofertilizers are liquid formulations containing the dormant form of desired microorganisms and their nutrients along with the substances that encourage formation of resting spores or cysts for longer shelf-life and tolerance to adverse conditions. The dormant forms, on reaching the soil, germinate to produce a fresh batch of active cells. These cells grow and multiply by utilizing the carbon source in the soil or from root exudates.

As an alternative to conventional carrier–based biofertilizers, liquid formulation technology, which has more advantages than the carrier-based inoculants, has been developed in the Department of Agricultural Microbiology, TNAU, Coimbatore. The advantages of liquid biofertilizers over conventional carrier-based biofertilizers are listed below:

- Longer shelf life, 12-24 months;
- No contamination;
- No loss of properties due to storage up to 45º C;
- Greater potential to fight with native populations;
- High populations can be maintained at more than 10^9 cells/ml up to 12 to 24 months;
- Easy identification by typical fermented smell;
- Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport;

- Quality control protocols are easy and quick;
- Better survival on seeds and soil;
- No need of running biofertilizer production units throughout the year;
- Very much easy to use by the farmer;
- Dosages are 10 times less than those of carrier-based powder biofertilizers;
- High commercial revenues;
- High export potential;
- Very high enzymatic activity, since contamination is nil.

Among different techniques to produce biofertilizer, the concept of effective microorganisms (EM), which are available in liquid form, was introduced in 1991 by Dr. Teruo Higa of Japan. The major groups of microorganisms contained in the EM include filamentous fungi, yeast, lactic acid bacteria and other soil bacteria. The application of EM aims to function as inoculum of microorganisms to the soil in which it will help to establish or re-establish soil ecosystems. EM is commercially available in concentrated form that needs to be processed before the application. According to the procedure suggested by the EM manufacturer, the concentrated EM (EM Bokashi) can be used directly by mixing with molasses and water. However, the common method is to use EM Bokashi as a starter to ferment the raw materials and produce either liquid or solid biofertilizer. The common raw materials include left-over plant or animal materials in the farms. The fermentation period was suggested to be at least seven days and the product is recommended to be used within three months. Today, the production of ready-to-use liquid biofertilizer from EM is becoming available in the market due to the convenience for small-scale farming or domestic application in which the users do not have space and raw materials available for fermentation.

There are three ways of using liquid biofertilizers.

- Seed treatment

  Seed treatment is the most common method adopted for all types of inoculants. The seed treatment is effective and economic. For a small quantity of seeds (up to 5 kg), the coating can be done in a plastic bag. For this purpose, a plastic bag sized 21” x 10” or larger can be used. The bag should be filled with 2 kg of seeds or more. The bag should be closed in such a way so as to trap the air as much as possible. The bag should be squeezed for 2 minutes or more until all the seeds are uniformly wetted. Then the bag is opened, inflated again and shaken gently. The shaking should stop after each seed gets a uniform layer of culture coating. The bag is opened and the seeds are shade-dried for 20–30 minutes. For large amounts of seeds, coating can be done in a bucket and the inoculant can be mixed directly by hand. Seed treatment with *Rhizobium, Azotobacter, Azospirillum*, along with PSM can be done. The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things that have to be kept in mind are that the seeds must be coated first with *Rhizobium, Azotobacter* or *Azospirillum*. When each seed gets a layer of these bacteria, then the PSM inoculant has to be coated as an outer layer. This method will provide maximum cell counts of all bacteria required for better results.

Treatments of seeds with any two bacteria will not provide a maximum number of bacteria on individual seeds.

- Root dipping

This method is used for application of *Azospirillum*/ /PSM on paddy transplating/ vegetable crops. The required quantity of *Azospirillum*/ /PSM has to be mixed with 5–10 litres of water at one corner of the field and the roots of seedlings has to be dipped for a minimum of half an hour before transplantation.

- Soil application

Use 200 ml of PSM per acre. Mix PSM with 400 to 600 kg of cow dung farmyard manure along with ½ bag of rock phosphate, if available. The mixture of PSM, cow dung and rock phosphate has to be kept under any tree or in the shade overnight and 50% moisture should be maintained. The mixture is used for soil application in rows or during leveling of soil.

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