TRENDS IN BIOFERTILIZERS PRODUCTION

Contents

INTRODUCTION

Biofertilizers hold a promising future in the development of the market, production, technologies, tools and instruments etc. They are promising in reducing soil quality problems with optimum crop yield. As it was highlighted in Part I of Module 1, biofertilizers are a complex product of live microbial inoculants which are able to fix atmospheric nitrogen, solubilize soil phosphorus, decompose organic material or oxidize sulphur in the soil. Biofertilizers are artificially multiplied cultures of beneficial soil microorganisms that can improve soil fertility and crop productivity. They add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth-promoting substances. They are made from biological wastes and do not contain any chemicals. The main sources of biofertilizers are bacteria, fungi and cynobacteria (blue-green algae).

TRENDS

Development of new eco-friendly technologies for production

The new eco-friendly technologies for production of biofertilizers will overcome the shortcomings of the conventional chemical-based farming which dominates at present. The implementation of technologies shows positive influence on both soil sustainability and plant growth. They support and gradually improve soil fertility by fixing atmospheric nitrogen. They increase the phosphorous content of the soil by solubilizing and releasing unavailable phosphorous. They participate in restoring depleted nutrients in the soil. Growth-promoting substances released by biofertilizers improve plant root proliferation. They also guard the plant against some soil-borne diseases. To popularize and implement more biofertilizers, there is a need of development of new technologies as follows:

Correct soil treatment

The role of plant nutrients in crop production is well-established and 16 essential plant nutrients have to be available to the crops in required quantities to achieve the yield target. Many studies have also emphasized the importance of N, P and K in enhancing the natural ability of plants to resist stress from drought and cold, pests and diseases. The essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients.

It is necessary to assess the capacity of a soil to supply the lacking amounts of needed plant nutrients (total crop requirement–soil supply). This is also important to produce a good biofertilizer formulation and to supply nutrients that can improve soil health and plant fertility. Several authors have focused their attention on the potential usage of nitrogen from animal manures. Nonetheless, the effort to find a source alternative to animal manure needs further study. Granite powder has also been studied as a good source of slow-release K fertilizer.

Generally, the addition of nitrogen to high C:N ratio residues is capable of accelerating the microbial activity during the fermentation process.

The number of microorganisms and the level of macro- and micronutrients obviously affect the growth of plants. One of the benefits of fertilizers is that they contribute to the availability of the microorganism population. Having a higher initial count of appropriate microbes in a ready biofertilizer right after the fermentation is essential. One of the ways to increase the number of selected microorganisms is by using the concept of an effective microorganism (EM) as introduced by Higa and Wididana (1991). Field experiments are needed to determine the nutrient availability and efficacy of most organic fertilizers. Such an experiment is important because the nutrient content of organic fertilizers varies widely. The quality is directly determined by the number of selected microorganisms in an active form per gram and their capability to promote plant growth and soil fertility.

Water-in-oil emulsions appear to be a good, yet underutilized, method for storing and delivering microorganisms through liquid formulations. The oil traps the water around the organism and, therefore, slows down water evaporation once applied. This is particularly beneficial for organisms that are sensitive to desiccation or in the case of use for horticultural crops where irrigation systems are in place. Water-in-oil emulsions allow the addition of substances to the oil and/or aqueous phases which could improve both the cell viability and the kinetics of release. However, cell sedimentation during storage is a major issue to be considered. *Studies aimed at solving this problem with the help of nanomaterials are underway*. Thickening the oil phase using hydrophobic silica nanoparticles can significantly reduce cell sedimentation and improve cell viability during storage.

Preparation of bacterial inoculants is supported by implementation of a new process based on the application of supercritical fluid properties which has been tested to encapsulate virus formulations. The process, named PGSS (Particles from Gas Saturated Solutions), is carried out at low temperatures and uses carbon dioxide as a supercritical fluid. Therefore, there should be no negative effects on the microbial viability, and the cost of production would be relatively low. The final product of the process is almost spherical particles that form a free-flowing powder which can be suspended in water. The possibilities of the PGSS process have already successfully been demonstrated for several solids and liquids.

Another interesting new technology is the exploitation of the natural production of bacterial biofilms as a possible carrier, and not only for the production of the inoculum, of defined bacterial or fungal–bacterial consortia. Biofilm production is already obtained for different industrial applications (e.g., wastewater treatment, production of chemical compounds). Two types of biofilms are employed in that case: biofilms growing onto inert supports (charcoal, resin, concrete, clay brick, and sand particles) and biofilms that are formed as a result of aggregate formation. In the first case, biofilms grow all around the particles, and the size of the biofilm particles grows with time usually to several millimeters in diameter. Biofilms formed by aggregation are called granular biofilms; granule formation may take from several weeks to several months.

There are four stages to the development of a mature biofilm: initial attachment, irreversible attachment by the production of EPS, early development, and maturation of biofilm architecture. What is particularly critical is the production of EPS, which serves to bind the cell to the surface and to protect it from the surrounding environment. EPS can be composed of polysaccharides, proteins, nucleic acids or phospholipids. A common EPS produced by bacterial cells in biofilms is the exopolysaccharide alginate. Beneficial biofilms developed in *in vitro* cultures containing both fungal and bacterial strains have been used as biofertilizers for nonlegume species with good efficacy. Application of a biofilmed inoculant containing a fungal-rhizobia consortium significantly increased N_2 fixation in soybean compared to a traditional rhizobium inoculant. Wheat seedlings inoculated with biofilm-producing bacteria exhibited an increased yield in moderate saline soils. Biofilms seem also to help the microorganisms to survive after inoculation even under stress conditions: this is a key aspect for the effectiveness of PGPM inoculation under agricultural conditions. Inoculants made with biofilms were shown to allow their rhizobia to survive at high salinity (400 mM NaCl) by 105-fold compared to rhizobial monocultures. Interestingly, beneficial endophytes were observed to produce higher acidity and plant growth-promoting hormones than their mono- or mixed cultures with no biofilm formation.

Technologies used for the production of living hybrid materials could be a new frontier in the development of carriers for PGPMs. Silica has appeared as a promising host for microorganism encapsulation: immobilization pathways are based on immobilization of a population of bacteria dispersed into a silica gel. Bacteria can be either entrapped into alginate microbeads coated with silica membranes or into macrocavities created inside the silica matrix. Such materials improve the mechanical properties of the alginate bead, the reduce cell leakage and enhance the cell viability.

The application of bio-nanotechnology could also provide new avenues for the development of carrier-based microbial inoculants. Nanotechnology employs nanoparticles which are made of inorganic or organic materials that are defined by having one or more dimensions in the order of 100 nm or less. The integration of whole cells with nanostructures leads to hybrid systems that have numerous applications in many fields, including agriculture. Indeed, even though nanoscale constructs are smaller than cells, macroscopic filters, made of radially aligned carbon nanotube walls, able to absorb *Escherichia coli*, were fabricated. The same technology could therefore be applied to collect bacterial cells from fermentation processes and deliver them to the plant. The physical stability and the high surface area of nanotubes, together with the ease and cost-effective fabrication of nanotube membranes may thus expand their use in the production of biofertilizer. The use of nanoformulations may enhance the stability of biofertilizers and biostimulators with respect to desiccation, heat and UV inactivation. The addition of hydrophobic silica nanoparticles of 7–14 nm to the water-in-oil emulsion formulation of the biopesticide fungus *Lagenidium giganteum* reduced the desiccation of the mycelium. The physical features of the formulation were improved and the microorganism was still effective after 12 weeks of storage at room temperature.

PRODUCT MODIFICATION AND INTRODUCTION OF INNOVATIVE PRODUCTS

The basic need of modern marketing is to regularly keep track of the consumers behaviour and adapt immediately to the requirements or the benefits sought by the consumers. As far as biofertilizers are concerned, it has been consistently argued for over a decade that there are tremendous product- and market-related constraints; however, the marketing organizations have not been able to adapt to the needs of the business environment.

The biofertilizers in a powder form have several constraints, as discussed above, which could be overcome to a great extent by product modification from a "powder form" to a "liquid form", which has tremendous superior benefits, as discussed below. The product innovation is another step forward towards tackling farmers' issues and some of them are the potash mobilizers like *Frateuria aurentia*, zinc and sulphur solubilizers like *Thiobacillus* species and manganese solubilizer fungal cultures like *Penicillium citrinum*, which have been identified for commercial operations and are highly useful and economical for enhancing agricultural productivity.

DEVELOPMENT OF BIOFERTILIZERS LEGISLATION

There are no specific regulations in the European Union that set parameters for biofertilizers. Each country locally regulates this matter. For example, the Polish Law on Fertilizers and Fertilization of July 10th 2007 includes "growth stimulators" in the category of plant conditioners. These are products which have "a positive impact on plant growth or other metabolic processes of plants in other ways than plant nutrients" and shall "pose no threat to [the] health of humans or animals or to the environment after their use according to use and storage instruction". This definition can be applied to biofertilizers, but no specific requirements are foreseen for such a category of products.

Spain, which is the second largest producer of conventional fruit and vegetables after Italy and among the leading countries in organic crops in Europe, does not include the term 'biofertiliser' in its legislation. The newest legal provision dealing with fertilizers (Real Decreto 506/2013) defines the number of microorganisms in organic amendments and compost but does not mention plant beneficial microorganisms. Fertilizers are defined as "Products used in agriculture or gardening, which, for their nutrient content, facilitate plant growth, increase performance and improve crop quality or which, by their specific action, amending, as appropriate, modify soil fertility or its physical, chemical or biological properties and that meet the requirements of Article 4.2 of this Royal Decree characteristics." Fertilizers, specialty products and amendments are also included in this definition. The Spanish administrative system allows local administrations to additionally regulate the matter (http://www.juntadeandalucia.es).

In Italy, only the mycorrhizal fungi inoculants are included within the group of "Products with action on the soil" and in the miscellaneous category of "Products with specific action" foreseen in the Decreto Legislativo of 29th April 2010, n. 75. The quality requirements established by the legal provision foresee that the inoculum is reproduced under sterile conditions on roots of sorghum in a substrate formed by an organic soil conditioner and rhizosphere bacteria. These conditions, particularly the "sterile conditions" requirement, are practically very difficult to achieve, considering the need of organic substrate. Besides, the presence of rhizosphere bacteria requires, from the point of view of the mycorrhizal fungus, unsterile conditions of the substrate. The label of such products shall indicate which organic matrix is used (presumably as a carrier), the name of the mycorrhizal fungal species included, and the name of rhizosphere bacteria and trichoderma species, even though the last two types of microorganisms are not AMF. No genetically modified organisms are allowed to be utilized for making this product; pathogens such as *Salmonella* spp., *Escherichia coli*, and other aerobic mesophilic microorganisms and nematode eggs shall not be present.

Proposals for an EU legislation on biofertilisers

The overall EU policy for the development of the agricultural sector in the next programming period (EU COM (2020)) underlines the need of reducing the impact on the environment of agricultural practices and the possibility of an increased use of alternatives to chemical inputs. The achievement of the objectives of rural development, which contribute to the Europe 2020 strategy for smart, sustainable and inclusive growth shall be pursued, among others, through the improvement of soil management, the preservation of biodiversity, the fostering of knowledge transfer and innovation and the promotion of resource efficiency. Furthermore, there is a strong emphasis on a wider application of agricultural practices based on low input (e.g. EU Directive 2009/128 on the sustainable use of pesticides) and on organic farming practices. Based on these policies, the support to research dedicated to biotechnological processes and products has a strong focus through the Horizon 2020 Programme (EU COM (2011) 808). In such a context, it is thus feasible to expect an increased interest among producers to develop products based on biological compounds and microorganisms.

DEVELOPMENTS OF THE BIOFERTILIZERS MARKET

There is a nascent but aggressively growing biofertilizers market. Among the major concerns in today's world are the pollution and contamination of soil by excessive and injudicious use of agrochemicals, as well as their detrimental effects to humans, in particular, by agricultural workers and rural communities. The concerns on both the health and environmental front have compelled governments to look for environmentally friendly options and switching from 'risk reduction' and 'safe use' procedures, in sustainable agricultural production. The use of biofertilizers and biopesticides offers a better option to augment the ‘Fertilizer Use Efficiency’ and maintain soil health. Biofertilizers are seen as an important component in Integrated Nutrient Management, with a supplementary role for the largest consumers of fertilizers.

TRENDS IN BIOFERTILIZERS PRODUCTION

Challenges and options in the biofertilizer business

In spite of being a cost-effective input, biofertilizers have not been completely accepted by the farmers till now. Some of the reasons/constraints for this low acceptance of biofertilizers are narrated below. However, the product modification as a "liquid form" has overcome some limitations and has provided opportunities for marketers.

Marketing challenges:

a) Biofertilizers are live microorganisms which die in case of high temperature.

b) The shelf-life of biofertilizers is limited to 6–12 months in powder form.

c) Biofertilizers are used before sowing and delay in dispatches leads to inventory carry-over and expiry of product.

d) Some biofertilizers are crop specific as well as location specific and, therefore, their efficacy does not remain the same at different locations due to differences in agro-climatic conditions and soil edaphic factors.

e) Soil characteristics like high nitrate, low organic matter, less available phosphate, high soil acidity or alkalinity, high temperature as well as presence of high levels of agro-chemicals or low levels of micro-nutrients contribute to failure of inoculants or adversely affect their efficacy.

f) The changes in the cropping patterns by farmers also adversely affect the sales.

g) Supply of sub-standard or spurious material by some manufacturers also adversely affects the credibility of biofertilizers, as they are a new product.

h) Some firms are selling organic manures as biofertilizers. Some organizations state a shelf-life of two/one year despite the norm of maximum 3–6 months.

i) Naturally occurring soil microflora and fauna also often inhibit the growth of introduced inoculums due to competition.

j) Lack of awareness among farmers regarding the benefits of biofertilizers.

k) There is no magic effect of biofertilizers and their impact is not visible in standing crop and, therefore, farmers are not convinced with the benefits of biofertilizer use.

Trend option – switching over to liquid biofertilizers, as they are superior than powder-based ones

1. Longer shelf-life to as long as 12 to 24 months.

2. No contamination.

3. No effect of high temperature, as tolerant up to 45 degrees Celsius with no activity loss.

4. Greater potential to fight with native population.

5. High population density of more than 10^9 cells/mL can be maintained up to 12 to 24 months.

6. Easy identification by typical fermented smell.

7. Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport.

8. Quality control protocols are easy and quick.

9. Better survival on seeds and soil.

10. No need of running biofertilizer production units throughout the year.

11. Very easy to use by the farmer.

12. Dosage is 10 times less than that of carrier-based powder based biofertilizers.

13. High commercial revenues.

14. High export potential.

15. Very high enzymatic activity, since contamination is nil.

Some examples of marketing strategies, as suggested below, may work strongly in the marketing of biofertilizers:

1. Field demonstration.

The farmers do what they see because "seeing is believing" and, therefore, result as well as method demonstration is a very effective tool in promoting the use of biofertilizers. The producers may synergize their efforts on this front, as biofertilizers are new and it is very crucial to show the impact of biofertilizer use to farmers and educate them about the economics/returns. Therefore, a demonstration farm may be developed jointly, at different locations, defining a catchment area, which could be shown to farmers at different crop stages.

2. Market segmentation and product positioning.

The segmentation is primarily dividing the market into various groups of buyers. The biofertilizer market can be segmented by "specific crop grower (Fruits/ Vegetables/ Oilseed/ Pulses/ Sugarcane/ Cereals), institutional buyers (Cane / Tea / Coffee / cotton/ oilseeds/ pulses federations and research-farms, SFCI, Agro-industries, etc) and customer size (major/minor), geographical location (high/low-consuming area and accessibility), and product application (supplementary/exclusive)". Once the market is segmented, it is important to target the market and concentrate on the most profitable one. Positioning starts with a product, but positioning is not what one does to a product; rather, it is what one does to the mind of a prospective customer. Thus, the product is being positioned in the mind of the customer, i.e. how he/she perceives the product. In an "over-communicated society", the marketer must create distinctiveness. The appropriate

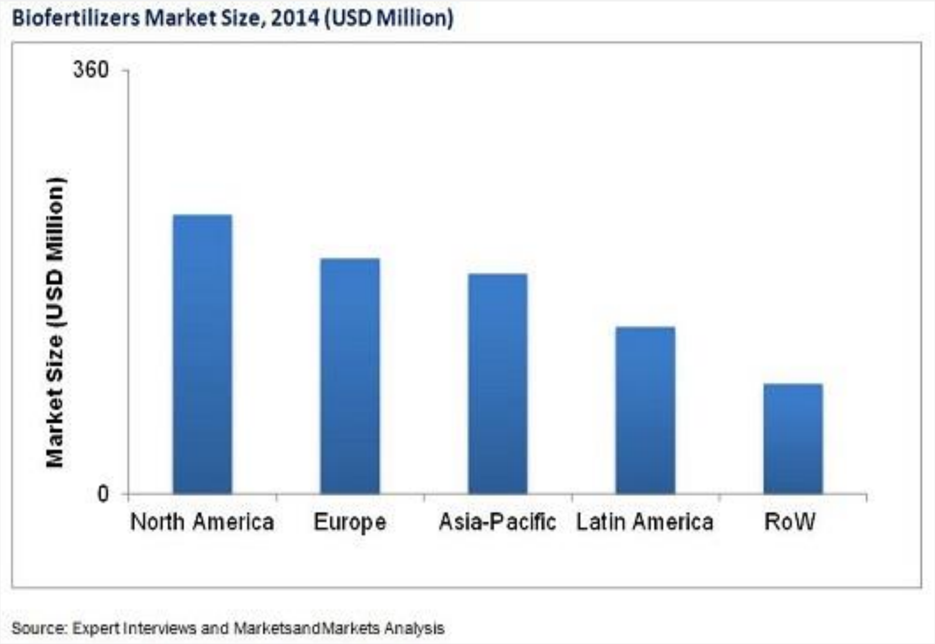
"USP" (Unique Selling Proposition) needs to be identified and propagated widely, for example: (a) Save cost through reduced dosage of chemical fertilizer; (b) Improves resistance power against disease; (c) Enhance sugar recovery percent in sugarcane.

Trends in pricing and sales promotion of right biofertilizers

Rural markets are quite "price sensitive" and particularly biofertilizers, being technical and new to farmers with a lot of constraints, do not fall under the category of "zero elasticity of demand" and need more push in view of lack of pull. The company generally determines the price of a product on the basis of its marketing objectives. Here, it is important to understand how biofertilizers are perceived in terms of value offered for money spent by customers. Biofertilizers have derived demand and so far, they have not really been perceived by farmers as giving those economic returns by reduction in the quantity of chemical fertilizers used. Unless farmers are convinced about substantial savings in cost of production through reduced usage of chemical fertilizers and getting similar yield, biofertilizer manufacturers will probably not be able to apply "pricing strategies".

The global biofertilizers market

The global biofertilizers market is expected to reach USD 1.88 billion by 2020 at a CAGR of 14.0% from 2015 to 2020. In 2012, the overall market was worth US $440.0 mln.



The biofertilizers market is projected to grow at a CAGR of 14.0% from 2015 to 2020. The increasing demand for organic products from emerging economies due to increased spending power and awareness level regarding health and wellness are expected to accelerate the growth of the biofertilizers market.

Sales and usage promotion

There is a great need to promote the product, from the point of view of both sales and usage. The channel members, i.e. dealer/distributors, need to be motivated by offering tangible benefits/incentives linking sales targets, such as “free family tour, gifts etc.” Similarly, the consumer also needs to be attracted by offers of coupons, premiums, contests, buying allowances etc. based on customer characteristics/buying behaviour. The progressive farmer village leaders, besides dealers, may also be identified for the purpose of conducting demonstrations and should be appropriately compensated.

Publicity and training

The POS (Point of Sales) material must be made available to all dealer/distributors and it also needs to be ensured that the product is displayed visibly. Wider publicity through Radio and educational films screening also needs to be taken up vigorously. Free distribution of biofertilizer during farmer meetings must be avoided. The orientation and training programmes for field sales force and dealers/distributors also need to be chalked out. There is a need of an exclusive team of Extension Executives for promoting biofertilizers with constant visits and developing a close connection with farmers and undertaking demonstrations with replication in nearby villages.

The major research focus is and should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems.

The most important and specific research needs, according to Swapna Latha Aggani from Kakatiya University, should highlight the following points:

1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops;

2. Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant microorganism symbiosis;

3. Study of microbial persistence of biofertilizers in soil environments under stressful conditions;

4. Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems;

5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation;

6. Establishment of legislation and strict regulation for quality control in markets and application.

TRENDS IN INNOVATIVE PRODUCTION OF BIOFERTILIZERS AS KEY PLAYERS IN SUSTAINABLE AGRICULTURE BY IMPROVING SOIL FERTILITY, PLANT TOLERANCE AND CROP PRODUCTIVITY

The microbiome: potential significance of beneficial microbes in sustainable agriculture

The rhizosphere, which is the narrow zone of soil surrounding plant roots, can comprise up to 10^{11} microbial cells per gram of root and above 30,000 prokaryotic species that, in general, improve plant productivity. The collective genome of the rhizosphere microbial community enveloping the plant roots is larger compared to that of plants and is referred to as microbiome, whose interactions determine the crop health in natural agro-ecosystems by providing numerous services to crop plants *viz.*, organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling, weed control and biocontrol.

The metagenomic study provides the individual, the core rhizosphere and endophytic microbiomes activity in *Arabidopsis thaliana* using 454 sequencing (Roche) of 16S rRNA gene amplicons. It has been proposed that exploiting tailor-made core microbiome transfer therapy in agriculture can be a potential approach in managing plant diseases for different crops. Rhizosphere microbial communities as an alternative to chemical fertilizers has become a subject of great interest in sustainable agriculture and biosafety programmes.

A major focus in the coming decades would be on safe and eco-friendly methods by exploiting the beneficial microorganisms in sustainable crop production. Such microorganisms, in general, consist of diverse naturally occurring microbes whose inoculation into the soil ecosystem advances soil physicochemical properties, soil microbial biodiversity, soil health, plant growth and development and crop productivity. The agriculturally useful microbial populations cover plant growth-promoting rhizobacteria, N_2-fixing cyanobacteria, mycorrhiza, plant disease suppressive beneficial bacteria, stress-tolerant endophytes and biodegrading microbes. Biofertilizers are a supplementary component to soil and crop management traditions, *viz.* crop rotation, organic adjustments, tillage maintenance, recycling of crop residue, soil fertility renovation and the biocontrol of pathogens and insect pests, whose operation can be significantly useful in maintaining the sustainability of various crop productions. *Azotobacter*, *Azospirillum*, *Rhizobium*, cyanobacteria, phosphorus- and potassium-solubilizing microorganisms and mycorrhizae are some of the PGPRs that have been found to increase in the soil under no tillage or minimum tillage treatment. Efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* can provide significant amount of nitrogen to *Helianthus annus* and to increase the plant height, number of leaves, stem diameter percentage of seed filling and seed dry weight. Similarly, in rice, addition of *Azotobacter*, *Azospirillum* and *Rhizobium* promotes the physiology and improves the root morphology.

Azotobacter plays an important role in the nitrogen cycle in nature, as it possesses a variety of metabolic functions. Besides playing a role in nitrogen fixation, *Azotobacter* has the capacity to produce vitamins, such as thiamine and riboflavin, and plant hormones, *viz.* indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). *A. chroococcum* improves the plant growth by enhancing seed germination and advancing the root architecture by inhibiting the pathogenic microorganisms around the root systems of crop plants. This genus includes diverse species, namely, *A. chroococcum, A. vinelandii, A. beijerinckii, A. nigricans, A. armeniacus* and *A. paspali*.

It is used as a biofertilizer for different crops, *viz.* wheat, oat, barley mustard, sesame, rice, linseeds, sunflower, castor, maize, sorghum, cotton, jute, sugar beets, tobacco, tea, coffee, rubber and coconuts. *Azospirillum* is another free-living, motile, Gram-variable, aerobic bacterium that can thrive in flooded conditions and promotes various aspects of plant growth and development. *Azospirillum* has been shown to exert beneficial effects on plant growth and crop yields both in greenhouse and in field trials. Diverse species of the *Azospirillum* genus, including *A. lipoferum*, *A. brasilense, A. amazonense, A. halopraeferens* and *A. irakense* have been reported to improve the productivity of various crops. Interestingly, it was observed that *Azospirillum* inoculation can change the root morphology via producing plant growth-regulating substances via siderophore production. It also increases the number of lateral roots and enhances the formation of root hairs to provide more root surface area to absorb sufficient nutrients. This improves the water status of the plant and aids the nutrient profile in the advancement of plant growth and development. Co-inoculation of *Azospirillum brasilense* and *Rhizobium meliloti* plus 2,4-D had a positive effect on the grain yield and N, P, K content of *Triticum aestivum*. *Rhizobium* has been used as an efficient nitrogen fixer for many years. It plays an important role in increasing yields by converting atmospheric nitrogen into usable forms. Being resistant to different temperature ranges, *Rhizobium* normally enters the root hairs, multiplies there and forms nodules. *Rhizobium* inoculants in different locations and soil types have been reported to significantly increase the grain yields of Bengal gram and lentil and enhance the rhizosphere of pea, alfalfa and sugar beet, berseem, ground nut and soybean. *Rhizobium* isolates obtained from wild rice have been reported to supply nitrogen to the rice plant to promote growth and development. A Rhizobiaceae species, *Sinorhizobium meliloti* 1021, infects plants other than legumes, e.g. rice, to promote growth by enhancing the endogenous level of plant hormone and photosynthesis performance to confer plant tolerance to stress. In groundnut, the IRC-6 rhizobium strain has resulted in the enhancement of several useful traits such as increased number of pink coloured nodules, nitrate reductase activity and leghaemoglobin content in 50 DAI (days after inoculation). Rhizobial symbiosis provides defence to plants against pathogens and herbivores, such as, Mexican bean beetle and the greenhouse whitefly *Trialeurodes vaporariorum*.

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Potential use of soil microbes in sustainable crop production

The beneficial soil microorganisms sustain crop production either as biofertilizers or as symbionts. They perform nutrient solubilization, which facilitates the nutrient availability and thereby uptake. This improves the plant growth by advancing the root architecture. Their activity provides several useful traits to plants such as increased root hairs, nodules and nitrate reductase activity, and efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* can provide a significant amount of available nitrogen through nitrogen cycling. Biofertilizers produce plant hormones, which include indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). Biofertilizers improve photosynthesis performance to confer plant tolerance to stress and increase the resistance to pathogens, thereby resulting in crop improvement.

Biofertlizers exploitation and nutrient profile of crops

A key advantage of beneficial microorganisms is to assimilate phosphorus for their own requirements, which in turn, becomes available in its soluble form in sufficient quantities in the soil. *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Fusarium, Sclerotium, Aspergillus* and *Penicillium* have been reported to be active in the solubilization process. A phosphate-solubilizing bacterial strain NII-0909 of *Micrococcus* sp. has polyvalent properties, including phosphate solubilization and siderophore production. Similarly, two fungi, *Aspergillus fumigatus* and *A. niger*, isolated from decaying cassava peels have been found to convert cassava wastes by the semi-solid fermentation technique to phosphate biofertilizers. *Burkholderia vietnamiensis*, a species of stress tolerant bacteria, produces gluconic and 2-ketogluconic acids, which are involved in phosphate solubilization. *Enterobacter* and *Burkholderia* isolated from the rhizosphere of sunflower produce siderophores and indolic compounds (ICs) which can solubilize phosphate. Potassium-solubilizing microorganisms (KSM), such as the genera *Aspergillus*, *Bacillus* and *Clostridium*, are efficient in potassium solubilization in the soil and mobilization in different crops. Mycorrhizal mutualistic symbiosis with plant roots satisfies the plant nutrients demand, which leads to enhanced plant growth and development, and protects plants from pathogen attacks and environmental stress. It leads to the absorption of phosphate by the hyphae from outside to the internal cortical mycelia, which finally transfer phosphate to the cortical root cells. Nitrogen-fixing cyanobacteria, such as *Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena* and *Plectonema*, are commonly used as biofertilizers. Besides the contribution of nitrogen, growth-promoting substances and vitamins liberated by these algae, *Cylindrospermum musicola* increases the root growth and yield of rice plants. Interestingly, genetic engineering was used to improve the nitrogen-fixing potential of *Anabaena* sp. strain PCC7120. Constitutive expression of the *hetR* gene driven by a light-inducible promoter enhanced HetR protein expression, leading to higher nitrogenase activity in *Anabaena* sp. strain PCC7120 as compared with the wild-type strain. This, in turn, caused better growth of paddy when applied to the fields.

Biofertilizers relevance and plant tolerance to environmental stress

Abiotic and biotic stresses are the major constraints that affect the productivity of crops. Many tools of modern science have been extensively applied for crop improvement under stress, of which the role of PGPRs as bioprotectants has become of paramount importance in this regard. *Trifolium alexandrinum* inoculated with *Rhizobium trifolii* showed higher biomass and increased nodulation under salinity stress conditions. *Pseudomonas aeruginosa* has been shown to withstand biotic and abiotic stresses. Paul and Nair found that *P. fluorescens* MSP-393 produces osmolytes and salt-stress induced proteins that overcome the negative effects of salt. *P. putida* Rs-198 enhanced the germination rate and several growth parameters, *viz,* plant height, fresh weight and dry weight, of cotton under alkaline and high-salt conditions via increasing the rate of uptake of K^+, Mg^{2+} and Ca^{2+}, and by decreasing the absorption of Na^+. A few strains of *Pseudomonas* reportedly confer plant tolerance via 2,4-diacetylphloroglucinol (DAPG). Interestingly, systemic response was found to be induced against *P. syringae* in *Arabidopsis thaliana* by *P. fluorescens* DAPG. Calcisol produced by PGPRs, *viz. P. alcaligenes* PsA15, *Bacillus polymyxa* BcP26 and *Mycobacterium phlei* MbP18, provides tolerance to high temperatures and salinity stress. It has been demonstrated that inoculation of plants with AM fungi also improves plant growth under salt stress. *Achromobacter piechaudii* was also shown to increase the biomass of tomato and pepper plants under 172 mM NaCl and water stress. Interestingly, a root endophytic fungus *Piriformospora indica* was found to defend its host plants against salt stress. It has been found that inoculation of PGPR alone or along with AM like *Glomus intraradices* or *G. mosseae* resulted in better nutrient uptake and improvement in the normal physiological processes in *Lactuca sativa* under stress conditions. The same plant treated with *P. mendocina* increased its shoot biomass under salt stress. Studies on the mechanisms involved in osmotic stress tolerance employing transcriptomic and microscopic strategies have revealed a considerable change in the transcriptome of *Stenotrophomonas rhizophila* DSM14405^T in response to salt stress. A combination of AM fungi and N_2-fixing bacteria helped the legume plants in overcoming drought stress. The effect of *A. brasilense* along with AM can be seen in other crops such as tomato, maize and cassava. *A. brasilense* and AM in combination improved the plant tolerance to various abiotic stresses. The additive effect of *Pseudomonas putida* or *Bacillus megaterium* and AM fungi was effective in alleviating drought stress. Application of *Pseudomonades* sp. under water stress improved the synthesis of antioxidant and photosynthetic pigments in basil plants. Interestingly, a combination of three bacterial species caused the highest CAT, GPX and APX activity and chlorophyll content in leaves under water stress. *Pseudomonas* spp. was found to have a positive effect on the seedling growth and seed germination of *A. officinalis* L. under water stress. The photosynthetic efficiency and the antioxidant response of rice plants subjected to drought stress have been found to increase after inoculation of arbuscular mycorrhiza. The beneficial effects of mycorrhizae have also been reported under both drought and saline conditions. Heavy metals such as cadmium, lead and mercury from hospital and factory waste accumulate in the soil and enter plants through the roots. *Azospirillium* spp., *Phosphobacteria* spp. and *Glucanacetobacter* spp. isolated from the rhizosphere of rice fields and mangroves have been found to be more tolerant to heavy metals, especially iron. *P. potida* strain 11 (P.p.11), *P. potida* strain 4 (P.p.4) and *P.*

fluorescens strain 169 (P.f.169) can protect canola and barley plants from the inhibitory effects of cadmium via IAA, siderophore and 1-aminocyclopropane-1-carboxylate deaminase (ACCD). It has been reported that rhizoremediation of petroleum contaminated soil can be expedited by adding microorganisms in the form of effective microbial agent (EMA) to different plant species such as cotton, ryegrass, tall fescue and alfalfa.

PGPRs as biological agents proved to be one of the alternatives of chemical agents to provide resistance to various pathogen attacks. Apart from acting as growth-promoting agents, they can provide resistance against pathogens by producing metabolites. *Bacillus subtilis* GBO3 can induce defense-related pathways, *viz.* salicylic acid (SA) and jasmonic acid (JA). Application of PGPR isolates, *viz. B. amyloliquefaciens* 937b and *B. pumilus* SE-34, provides immunity against tomato mottle virus. *B. megaterium* IISRBP 17 characterized from black pepper stem acts against *Phytophthor capsici*. *Bacillus subtilis* N11 along with mature composts was found to control *Fusarium* infestation on banana roots. Similarly, *B. subtilis* (UFLA285) was found to provide resistance against *R. solani* and also to induce foliar and root growth in cotton plants. In another interesting study, *Paenibacillus polymyxa* SQR-21 was identified as a potential agent for the biocontrol of *Fusarium* wilt in watermelon. Further, the exploitation of PGPRs was found to be effective to manage the spotted wilt viruses in tomato, cucumber mosaic virus of tomato and pepper, and banana bunchy top virus in banana. In some cases, along with bacteria, mycorrhizae can also confer resistance to fungal pathogens and inhibit the growth of many root pathogens, such as *R. solani*, *Pythium* spp., *F. oxysporum*, *A. obscura* and *H. annosum*, by improving the plant nutrient profile and thereby the productivity. For instance, *Glomus mosseae* is effective against *Fusarium oxysporum* f. sp. *basilica*, which causes root-rot disease of basil plants. *Medicago tranculata* also showed induction of various defense-related genes with mycorrhizal colonization. It was shown that addition of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* to the soil can reduce the development of root-rot disease and enhance the yield of *Phaseolus vulgaris* L.

Mechanism of action of various biofertilizers

Mycorrhiza is the association of fungi with the roots of higher plants. While it remains an enigma, it serves as a model system to understand the mechanism behind stimulation of growth in the root cells as a result of mycorrhizal inhabitation. The genome sequencing of two EM fungi (ectomycorrhizae), *L. bicolor* 13 and *T. melanosporum* (black truffle) 14, has helped in the identification of factors that regulate the development of mycorrhiza and its function in the plant cell. Fifteen genes up-regulated during symbiosis have been identified as putative hexose transporters in *L. bicolor*. Its genome lacks genes encoding invertases, making it dependent on plants for glucose. However, *T. melanosporum* possesses one invertase gene, and unlike *L. bicolor*, it can directly use the sucrose of the host. The up-regulation of transporter genes during symbiosis indicated the role of transportation of useful compounds like amino acids, oligopeptides and polyamines through the symbiotic interface from one organism to the other. Free-living mycelium can take nitrate and ammonium from the soil. Subsequently, these compounds reach the mantle and Hartig net and are then transferred to the plants. Cysteine-rich proteins (MISSP7) of the fungus play an important role as effectors and facilitators in the formation of symbiotic interfaces. Many genes related to auxin biosynthesis and root morphogenesis showed up-regulation during mycorrhizal colonization. Further, *G. versiforme* possesses inorganic phosphate (Pi) transporters on its hyphae, which help in the direct absorption of phosphate from the soil, and a glutamine synthase gene was found in *G. intraradice*, which strengthens the possibility that nitrogen metabolized in the fungal hyphae can be transported later to the plant. Bioactive compounds called Myc factors similar to the Nod factors of *Rhizobium* are suggested to be secreted by mycorrhiza and *Rhizobium* and to be perceived by host roots for the activation of signal transduction pathways or the common symbiosis (SYM) pathway. The pathways that prepare the plant for both AM and *Rhizobium* infection have some common points. The common SYM pathway prepares the host plant to bring about changes at the molecular and anatomical level with the first contact of fungal hyphae. So far, calcium is supposed to be the hub of secondary messengers via Ca^{2+} spiking in the nuclear region of root hairs. *Rhizobium leguminosarum* biovar *viciae* can induce various genes in plants like pea, alfalfa and sugar beet, as evident from the microarray studies. PGPRs produce IAA which, in turn, induces the production of nitric oxide (NO), which acts as a second messenger to trigger a complex signaling network leading to improved root growth and developmental processes.

Expression of ENOD11 and many defense-related genes and root-remodelling genes get up-regulated during entry. Subsequently, this allows the formation of a pre-penetration apparatus (PPA). Although the biology behind the development of arbuscules is unknown, a gene called *Vapyrin*, when knocked down, causes a decline in the growth of arbuscules. Many other genes, including those encoding subtilisin protease, phosphate transporter or two ABC transporters, are known to be involved in arbuscule formation. Nitrogen-fixation genes are popularly used by scientists today to create engineered plants that can fix atmospheric nitrogen. The induction of *nif* genes in case of nitrogen-fixing bacteria takes place under low concentration of nitrogen and oxygen in the rhizosphere. Interestingly, sugarcane plantlets inoculated with a wild strain of *G. diazotrophicus*, have demonstrated fixation of radioactive N_2 when compared with the *G. diazotrophicus* mutant that has a mutant *nifD* gene, which proved the significance of *nif* genes. The efficiency of nitrogen fixation is dependent on the utilization of carbon. Bacteria like *Bacillus subtilis* (UFLA285) can differentially induce 247 genes in cotton plants as compared to controls where no PGPR was supplied to the cotton plant. Many disease-resistance genes that work via jasmonate/ethylene signaling as well as osmotic regulation via proline synthesis genes were differentially expressed with UFLA285 induction. Various differentially expressed genes were identified, including ones encoding metallothionein-like protein type 1, a NOD26-like membrane integral protein, ZmNIP2-1, a thionin family protein, an oryzain gamma chain precursor, stress-associated protein 1 (OsISAP1), probenazole-inducible protein PBZ1, as well as auxin- and ethylene-responsive genes. The expression of the defense-related proteins PBZ1 and thionins have been found to get repressed in the rice–*H. seropedicae* association, suggesting the modulation of plant defense responses during colonization.

Among the PGPR species, *Azospirillum* has been suggested to secrete gibberellins, ethylene and auxins. Some plant-associated bacteria can also induce phytohormone synthesis. For example, lodgepole pine, when inoculated with *Paenibacillus polymyxa*, had elevated levels of IAA in the roots. *Rhizobium* and *Bacillus* were found to synthesize IAA at different cultural conditions such as pH, temperature and in the presence of agro-waste as a substrate. Ethylene, unlike other phytohormones, is responsible for the inhibition of growth of dicot plants. It was found by Glick *et al*. that PGPR could enhance the growth of the plant by suppressing the expression of ethylene. Interestingly, a model has been suggested in which ethylene synthesis from 1-aminocyclopropane-1-carboxylate (ACC), an immediate precursor of ethylene, which is hydrolyzed by bacterial ACC-deaminase enzyme in the need of nitrogen and carbon source is also one of the mechanisms of induction of conditions suitable for growth. ACC-deaminase activity has also been found in bacteria such as *Alcaligenes* sp., *Bacillus pumilus*, *Pseudomonas* sp. and *Variovorax paradoxus*. The involvement of ACC deaminase in the indirect influence on the growth of plants was proved in canola, where mutations in the ACC deaminase gene caused the loss of effect of growth-promoting *Pseudomonas putida*. Interestingly, the potential of PGPRs was further enhanced by introducing genes involved in the direct oxidation (DO) pathway and mineral phosphate solubilisation (MPS) into some useful strains of PGPRs. The gene encoding glucose dehydrogenase (*gcd*) involved in the DO pathway was cloned and characterized from *Acinetobacter calcoaceticus* and *E. coli* and *Enterobacter asburiae*. Moreover, a gene encoding a soluble form of GCD has been cloned from *Acinetobacter calcoaceticus* and *G. oxydans*. Furthermore, there are reports of site-directed mutagenesis of glucose dehydrogenase (GDH) and gluconate dehydrogenase (GADH) that has improved the activity of this enzyme. Mere substitution of S771M provided thermal stability to *E. coli*, whereas mutation of glutamate 742 to lysine improved the EDTA tolerance of *E. coli* PQQGDH. The application of this technology was achieved by transferring genes involved in the DO pathway, *viz. GDH, GADH* and pyrroloquinoline quinine (*PQQ*), to rhizobacteria and phosphoenolpyruvate carboxylase (*PPC*) to *P. fluorescens*, providing the MPS trait.

To recapitulate briefly, excess nutrients are accumulated in soils, particularly phosphorus, as a result of over-application of chemical fertilizers by farmers during intensive agricultural practices. The major research focus is and should be on the production of efficient and sustainable biofertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems.

Finally, let us reiterate the most important and specific points, as defined by Swapna Latha Aggani from Kakatiya University, on which the research on biofertilizers should focus:

1. Selection of effective and competitive multi-functional biofertilizers for a variety of crops;

2. Quality control systems for the production of inoculants and their application in the field, to ensure and explore the benefits of plant microorganism symbiosis;

3. Studies on microbial persistence of biofertilizers in soil environments under stressful conditions;

4. Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural production systems;

5. Transferring technological know-how on biofertilizer production to the industrial level and for optimum formulation;

6. Establishment of legislation and strict regulation for quality control in markets and application.

CONCLUSIONS

Environmental stresses are becoming a major problem and productivity is declining at an unprecedented rate. Our dependence on chemical fertilizers and pesticides has encouraged the thriving of industries that are producing life-threatening chemicals and which are not only hazardous for human consumption, but can also disturb the ecological balance. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realise the useful aspects of biofertilizers and implement their application to modern agricultural practices. The new technology developed using the powerful tool of molecular biotechnology can enhance the biological pathways of production of phytohormones. If identified and transferred to the useful PGPRs, these technologies can help provide relief from environmental stresses. However, the lack of awareness regarding improved protocols of biofertilizer applications to the field is one of the few reasons why many useful PGPRs are still beyond the knowledge of ecologists and agriculturists. Nevertheless, the recent progresses in technologies related to microbial science, plant–pathogen interactions and genomics will help to optimize the required protocols. The success of the science related to biofertilizers depends on invention of innovative strategies related to the functions of PGPRs and their proper application to the field of agriculture. The major challenge in this area of research lies in the fact that, along with the identification of various strains of PGPRs and their properties, it is essential to dissect the actual mechanism of functioning of PGPRs for their efficacy towards exploitation in sustainable agriculture.

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