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Shelf-life of Biofertilizers: An Accord between Formulations and Genetics

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To meet the steady demand of food supply, application of fertilizer is indispensable in modern agriculture. Role of fertilizers has already been proven by many countries with green revolution and by attaining food self-sufficiency within short period of time. Actually, application of synthetic/chemical fertilizers not only supplies essential nutrients to food crops but also provides them in an easily available manner. Therefore, these fertilizers can quickly enhance the growth and productivity of food crops and are quick to gain popularity. However, extensive use of such fertilizer leads to serious environmental concerns. Nitrate leaching and surface/ground water pollution due to increased use of fertilizer is directly related to human health problems. Similarly, freshwater contamination by chemical fertilizer/fertilizer residue is one of the major causes of eutrophication. Likewise, increased greenhouse gas emission as well as heavy metal uptake and accumulation by food crop could be considered as other environmental problems emerged due to synthetic fertilizers [1]. Moreover, chemical fertilizer could eliminate the beneficial microbial as well as insect community of soil. Alternatively, many of these problems can be surmounted by utilization of biofertilizers. It may not be a realistic idea to completely replace the chemical fertilizers by biofertilizer; however, biofertilizers have the potential to supplement the synthetic fertilizers and to significantly reduce its use.

In general, biofertilizers are living microorganisms, unlike chemical fertilizers; they themselves are not the source of nutrients but can help the plants in accessing the nutrient available in its surrounding environment. The microorganisms commonly used as biofertilizers may be nitrogen fixing soil bacteria (*Rhizobium, Azotobacter*), nitrogen-fixing cyanobacteria (*Anabaena*), phosphate solubilizing bacteria (*Pseudomonas putida*) and arbuscular mycorrhizal fungi. Similarly, phyto-hormone (auxin) producing bacteria and cellulolytic microorganisms could also be part of biofertilizer formulation. When applied to the field, the activities (nitrogen fixation, phosphate solubilization, production of phytohormones) of the plants are benefited resulting in improved growth and productivity. Therefore, viability of these organisms during production, formulation, storage, transportation/distribution and field application is directly related to plant growth promoting potential of a biofertilizer formulation. The complaint from farmers regarding the efficiency of biofertilizer is not uncommon and improper storage and longer duration between production and field application could be the best explanation for such incidents. This limits their use due to compatibility, stability and survival issues under different soil conditions. Hence, improved shelf life could be the key for further popularization of biofertilizer application.

Presently, a range of commercial biofertilizer formulations are available and different strategies have been applied to ensure maximum viability of the microorganisms used in such formulations. These strategies comprise: (i) optimization of biofertilizer formulation, (ii) application of thermo-tolerant/drought-tolerant/genetically modified strains and, (iii) application of liquid biofertilizer. For convenience of application, a carrier material is used as a vehicle for the microorganisms to be used as biofertilizer. Moreover, such materials may have a role in maintaining the viability (shelf-life) of the microorganisms prior to its release into the field as well as they also provide a suitable microenvironment for rapid growth of the organisms upon their release. A carrier could be a material, such as peat, vermiculite, lignite powder, clay, talc, rice bran, seed, rock phosphate pellet, charcoal, soil, paddy straw compost, wheat bran or a mixture of such materials. In common practice, for better shelf-life of biofertilizer formulation, a carrier or a mixture of such carrier materials are selected based on the viability of the microorganisms mixed with them. Similarly, pre-sterilization of the carrier material and its enrichment with nutrient is the other strategy to improve the shelf-life by allowing the microorganism to maintain/grow in a non-competitive microenvironment [2]. Sucrose, maltose, trehalose, molasses, glucose and glycerol are some supplementary nutrients and/cell protectants commonly used with a carrier material to ensure maximum cell viability and extended shelf-life.

Liquid biofertilizer formulation could be considered as one potential strategy for improving the shelf-life of biofertilizer. Unlike solid carrier based biofertilizers, liquid formulations allow the manufacturer to include sufficient amount of nutrients, cell protectant, and inducers responsible for cell/spore/cyst formation to ensure prolonged shelf-life. The shelf-life of common solid carrier based biofertilizers is around six months; however, it could be as high as two years for a liquid formulation [3,4]. Further, solid carrier based biofertilizers are less thermo-tolerant whereas; liquid formulations can tolerate the temperature as high as 55°C [3,5]. Hence, improved shelf-life could be achieved by the application of a liquid biofertilizer formulation. However, process cost of liquid biofertilizer is significantly higher than a solid formulation. Thus, successful commercialization of less expensive liquid biofertilizer is a challenge and shelf-life of such products is still a concern.

Consequently, efforts are also underway to conceive efficient biofertilizers compatible with a wide range of soils and plants by molecular and genetic engineering. For instance, biofertilizers have been produced, firstly, based on nitrogen-fixing rhizobial bacteria found "naturally" in the root nodules of legumes. However, these bacteria are not able to provide non-leguminous plants with the nitrogen that they fix from the atmosphere. In this case, molecular engineering is of particular interest, since it allows the development of effective delivery systems, so that non-leguminous plants can be grown with symbiotic rhizobial root nodules without the need for added nitrogen fertilizers [6].

Rhizobial biofertilizers are well-known for their potential to

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increase significantly plant yields under controlled conditions, but the strains fail to survive under certain soil conditions and they completely lose their ability to increase productivity. Since 1991, this has been associated with the weak competitiveness of rhizobial biofertilizers in relation to the indigenous soil microorganisms in the use of some nutrients. For example, some studies have shown that the failure to survive by the rhizobial strains, in the presence of other soil microorganisms is related to their inability to taking iron from soil. To improve their resistance, a gene increasing the iron use was isolated from another strain (*Bradyrhizobium japonicum*) and introduced into rhizobia [7]. Such genetic manipulation led to the growth stimulation. Thus, genetic manipulation is another way to enhance the stability of biofertilizers; nevertheless, it cannot totally replace the formulations as it would be a pre-step in production followed by formulation. Thus, a concerted effort between soil chemists, microbiologists, geneticists and agronomists is needed to facilitate enhanced shelf-life of biofertilizers to obtain better agricultural yields sustaining the environmental and human health.

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