Biotechnology: economic and social aspects

Issues for developing countries

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Biofertilizers: agronomic and environmental impacts and economics

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Introduction

During the last decades increased fertilizer and pesticide use contributed to a spectacular increase in crop production, especially in Asia and South America. However, the price of fossil-fuel-based inorganic fertilizers relative to the prices of most stable crops has increased and chemical pesticides are both costly and harmful when they persist in the soil and enter the food chain. This explains the emphasis on current attempts to control soil- and plant-associated microorganisms, to lower fertilizer production costs, reduce environmental pollution whilst ensuring fair or even high yields, and to expand the adaptability of plants to reputedly unfavourable situations. The approach adopted is to introduce into soil or rhizosphere soil symbiotic or non-symbiotic microorganisms, a practice known as inoculation. The inoculants are also known as biofertilizers. Inoculation of plants by beneficial bacteria or fungi is routinely used in the legume–rhizobia symbiosis, fairly often in the ectomycorrhizal and to some extent in the endomycorrhizal symbiosis. Recently inoculation of actinorhizal plants has been developed and successfully adopted both in temperate and tropical countries. With some exceptions, inoculation with plant-growth-promoting rhizobacteria (PGPR) is still in its experimental stage. Soil inoculation with free-living blue-green algae has been and is still practised in Southeast Asia but the results are irregular.

In this chapter the discussion is restricted (1) to the presentation of the main types of biofertilizers (exclusive of *Azolla* and other green manures) and their modes of action, (2) to their agronomic and environmental benefits, (3) to biofertilizer technology, and (4) to the economics of the application of biofertilizers. The use of chemicals of microbial origin such as antibiotics or toxins (e.g. toxins produced by *Bacillus thuringiensis*) is not dealt with.

Agronomic applications and environmental benefits

Agronomic applications

Biofertilizers are preparations containing viable forms of beneficial microorganisms intended for seed plant or soil application. They can affect growth and yield of plants either directly or indirectly through different mechanisms that are discussed later on.

The main groups of microorganisms brought to plants by biofertilizers are: *Rhizobium*, *Frankia*, mycorrhizal fungi, plant-growth-promoting rhizobacteria (PGPR), and blue-green algae (BGA).

Rhizobium Rhizobia provide the host plant with the N it requires for its growth. In association with legumes, rhizobia fix from 0 to 300 kg N_2 per ha per year, the percentage of N derived from nitrogen fixation in plant tissues ranging from 40 to 90%. In soils deficient in N, yield can be increased several-fold.

Inoculation should be limited to sites where it can be expected to generate a worthwhile response. A distinction should be made between the following situations.

When a non-promiscuous plant species is grown on a site for the first time it has to be inoculated with a highly performing specific strain. A response can be expected since non-promiscuous species can benefit from inoculation with certain specific strains, such as *Acacia mangium* inoculated with *Bradyrhizobium*.

By contrast, non- or less-promiscuous species (e.g. *Vigna unguiculata*, *Acacia crassiparpa*), that is species with no specific microbial requirements, would probably not benefit especially from inoculation since they can be infected by a large number of native symbiotic strains.

When growing a plant on a site where it had already been grown before, inoculation is often (though not always) useless, on account of the survival in soil of strains of symbiotic competent microorganisms.

Since there are several exceptions to these rules, it is necessary, prior to the start of any inoculation programme, to determine whether symbiotic strains are present in soil to be planted and to establish inoculation needs and proper management protocols.

Frankia About 200 plant species covering 19 genera and 8 families, known as actinorhizal plants, nodulate with an N_2-fixing actinomycete, *Frankia*. The main tropical species of actinorhizal plants belong to the genera *Casuarina*, *Allocasuarina*, *Gymnostoma* and *Alnus*. The amount of N_2 fixed annually by symbiotic systems (between a few kg per ha to 300 kg per ha) depends on the host plant, the symbiotic microorganism and the environmental conditions.

Reliable inoculants, namely those made of fresh or dried beads of *Frankia* entrapped in alginate (Sougoufara, Diem & Dommergues, 1989), have been developed and most satisfactory results obtained in the field. The limitations to the use of *Frankia* biofertilizers are similar to those underlined in the case of rhizobia.

Mycorrhizal fungi Mycorrhizal fungi are known to improve significantly plant productivity by enhancing the absorption of soil nutrients and water and by controlling certain soil pathogens and pests (Harley & Smith, 1983).

Precolonization of roots by mycorrhizal fungi frequently leads to reduced damage by soil-borne pathogens like *Fusarium*, *Phythium* or *Phytophthora* and nematodes. The mechanisms involved are not known but it has been suggested that mycorrhizal fungi may activate plant defence mechanisms or, in the case of ectomycorrhizae, act as a physical barrier or produce antimicrobial substances (Mosse, Stribley & Le Tacon, 1981; Gianinazzi, 1990).

Although more research is necessary for a better understanding of how mycorrhizae protect plants, these observations nevertheless open possibilities for associating the management of the mycorrhizae with a reduced input of xenobiotic substances in plant production.

Since the pioneer nursery trials on citrus (Menge, Lembright & Johnson, 1977) and several forest trees (Marx, 1980), the importance of mycorrhizae as potential biofertilizers for crops or trees has been established (Gianinazzi, Gianinazzi-Pearson & Trouvelot, 1990*a*; Marx & Cordell, 1990).

Even when beneficial effects are not so striking, mycorrhizae can ensure a decrease in the heterogeneity within a crop population in the field (Ganry *et al.*, 1982; 1985); they can also eliminate clone variability in nurseries (Blal, 1989).

The positive effect of mycorrhizae on yield varies in relation to the crop involved, the mycorrhizal fungi introduced or present, and the agricultural techniques used. In order to use mycorrhizal fungi successfully, it is necessary, as with other biofertilizers, to develop a strategy of inoculation based on biological tests (Gianinazzi, Gianinazzi-Pearson & Trouvelot, 1986) designed to evaluate: (1) the quantity of infective propagules present in a given soil (i.e. the soil mycorrhizal potential), (2) their effectiveness and potential impact on crop production, and (3) soil receptivity to mycorrhizal fungi used as inoculant for a given crop.

Plant-growth-promoting rhizobacteria (PGPR) These bacteria that colonize the plant rhizosphere, can enhance directly or indirectly plant productivity through different mechanisms, such as nitrogen fixation to some extent, solubilization of non-mobile soil nutrients such as phosphorus, production of phytohormones, antagonism against deleterious or pathogenic microorganisms, and degradation of phytotoxins (Lynch, 1983).

The impact of PGPR varies greatly with the bacteria used to inoculate the crops as is shown by the following examples.

(1) *Nitrogen-fixing PGPR* (also known as nitrogen-fixing associative rhizobacteria) have been extensively studied since 1970, the best known species being) *Azotobacter paspali*, *Azospirillum lipoferum*, *A. brasilense*, and *A. amazonense* (Dobereiner & Pedrosa, 1987). Others such as *Herbaspirillum seropedicae* (Dobereiner & Pedrosa, 1987) and *Saccharobacter nitrocaptans* (Cavalcante & Dobereiner, 1988) have been discovered more recently. Generally these bacteria contribute less than a few kilograms per hectare yearly, or very little nitrogen to their ecosystems. Bashan, Singh & Levanony (1989) demonstrated with a Nif^- strain that the contribution of *Azospirillum brasilense* to the improvement of tomato seedling growth is not through nitrogen fixation. However, when some of these bacteria are associated with specific host cultivars of plants such as sugarcane and *Panicum* sp., nitrogen fixation can become quite significant (Boddey, 1987).

(2) In the specific case of rice the general effect of bacterial inoculation on yield, as shown by the analysis of 210 experiments reported in 23 papers (Roger, Zimmerman & Lumpkin, 1991), is an average increase by 27.6% in pot experiments (87 data) and by 14.4% in field experiments (123 data). In the field, positive effects, no effect, inconsistent effects in time or among various simultaneous treatments, and negative effects were reported.

Differences in yield ranged from −25 to +69%. The current limiting factor for bacterial inoculation of rice is the lack of proven technology, which results from an insufficient knowledge of the factors that allow inoculated strains to establish and their mode of action on rice. Currently, most strains tested for inoculation have been nitrogen-fixing forms, but ARA, ^{15}N, and nitrogen balance studies did not show that the promotion of growth and nitrogen uptake was due to higher biological nitrogen-fixation (BNF). The beneficial effect of bacterial inoculation can be attributed to a combination of (1) increased BNF, (2) production of PGPR favouring rice growth and nutrient utilization, (3) increased nutrient availability through solubilization of immobilized nutrients by inoculated bacteria, and (4) competition of inoculated strains with pathogens or detrimental bacteria in the rhizosphere. The relative importance of these four components has not yet been explained. In the current status of knowledge, no definite conclusion regarding the potential of bacterial inoculation of rice can be drawn.

(3) Phosphorus-solubilizing microorganisms: according to Subba Rao (1986) inoculation of various crops with *Phosphobacterin* (biofertilizer containing phosphate-dissolving bacteria) could increase yield by 10–37%. However, results were irregular; improvements in cereal yield were found only in 10 out of 37 field experiments.

Experimental results on the effect of inoculation with these microorganisms in rice fields are inconsistent (Roger *et al.*, 1991).

(4) *Pseudomonas* used for biological control: *Pseudomonas* spp. can inhibit growth of deleterious and pathogenic rhizosphere bacteria and fungi by producing siderophores. These are peptides that have a strong specific affinity for iron and with which they form a stable complex. Thus iron becomes unavailable to soil microorganisms that do not produce siderophores or produce some with lower affinity for iron (Kerr, 1982). *Pseudomonas putida* produces a siderophore called pseudobactin that controls both potato soft rot and seed piece decay (Kloepper *et al.*, 1980). In the USA, seed treatment with plant-growth-promoting pseudomonads is becoming a standard commercial practice in wheat and barley monocultures subject to the take-all root disease caused by *Gaeumannomyces graminis* var. *tritici*. Both antibiotics and siderophores have been implicated in the control of take-all disease. Siderophores become ineffective in anaerobic conditions when formation of organic acids solubilizes the iron that they bind. Also, some target organisms show resistance when they acquire the siderophore system (Loper & Ishimaru, 1989).

Blue-green algae (BGA) BGA can improve rice yield not only by contributing to the nitrogen nutrition of the crop and, reportedly, by other effects including (1) production of plant growth regulators, (2) improvement of soil properties, (3) increased solubilization of phosphorus,

(4) decrease of weed incidence, and (5) alleviation of detrimental effects of sulphate reduction (Roger & Kulasooriya, 1980).

Nitrogen contribution to soil by BGA depends on the turnover of biomass, for which no data are available. Nevertheless, the observation that BGA usually bloom once or twice during a crop cycle indicates a rough potential of 30 kg N per ha per crop. The maximum theoretical N contribution, calculated by assuming that all carbon input in the photic zone is through nitrogen-fixing BGA, is 75 kg per ha per crop. Estimates of photo-dependent BNF in ricefields range from a few to 80 kg N per ha per crop (average 27 kg). Estimates in 65 plots of an IRRI farm during four crops ranged from 0 to 55 kg N per ha per crop, and averaged 19 kg in no-N controls, 8 kg in plots with broadcast urea, and 12 kg in plots where urea was deep-placed. Experimental recoveries of BGA nitrogen by rice ranged from 13 to 50% (average 30%), and were dependent on the use and incorporation or not of fresh or dried material and on the occurrence of soil fauna.

In a bibliographic survey covering 634 field experiments inoculation of rice was found to have induced an increase of grain yield of 257 kg per ha. However, only 17% of the experiments reported statistically significant differences. Though algal inoculation can increase rice yield its effects often seem to be erratic and low. This may be the reason for its limited adoption and use.

Environmental benefits

Biofertilizers, in addition to their impact on plant productivity, contribute to the sustainability of soil fertility and to a reduction of the hazards of pollution. An example of the beneficial effect of biofertilizers on the maintenance of soil fertility is that of the binding role of mycorrhizal hyphae that bind soil aggregates together, strengthen soil structure and reduce soil erosion (Allen & Macmahon, 1985). In contrast, the excessive use of soluble chemical fertilizers, with their content of phosphate and nitrate, is one of the main causes of water pollution.

Inoculation practices of legumes and actinorhizal plants with their nitrogen-fixing symbionts drastically reduce and may suppress the utility of chemical N fertilizers. Such practice minimizes the pollution of the soil and water tables by nitrates and a number of other concomitant toxic compounds resulting from the use of non-biological fertilizers.

Blal *et al.* (1990) recently showed that VA endomycorrhizae greatly increase the fertilizer utilization coefficient (2.7–5.6 fold) as much of rock phosphate as of superphosphate for plants growing in acid, P-fixing soils. Under these conditions, VA fungi greatly contribute to optimizing phosphate fertilizer efficiency, so minimizing their input, and to utilizing natural, cheaper substitutes for plant fertilization. Although similar work has not been done with other type of mycorrhizae, there is some evidence that they may favour phosphate mobilization by plants from condensed or complex forms of phosphate (Gianinazzi-Pearson & Gianinazzi, 1989). All results emphasize the potential of these biofertilizers in reducing fertilizer costs and pollution in agriculture.

Biofertilizer technology

Culture of the selected microorganisms

The selected microorganism which has been screened or engineered for its symbiotic performance must be cultured in large quantities to produce enough inoculant for the field. Some microorganisms are easily grown *in vitro*, e.g. rhizobia and ectomycorrhizal fungi. Others, however, are much more difficult to produce in large quantities, e.g. *Frankia*, and, some have not yet been grown *in vitro*, viz. VAM fungi.

Rhizobium is easily grown in many types of fermentors, e.g. batch or semicontinuous culture (Williams, 1984). *Frankia* is much more difficult to grow *in vitro*. To offset this drawback, a reliable culture method has now been developed (Diem & Dommergues, 1989).

Efficient fungi forming ectomycorrhizae, ericoid and orchid endomycorrhizae can be easily cultured on solid and in liquid media. Production of large quantities of inoculum poses no real technical problems. Commercial inoculum of ectomycorrhizal fungi is now produced by the private sector in USA, Canada and France. In New Zealand, the commercial production of inoculum for ericoid endomycorrhizae is under way (Mintech Ltd). Commercial ectomycorrhizal inocula can be applied either in a liquid form to the field or incorporated into potting mixes for nursery plants.

Attempts to grow VA endomycorrhizal fungi in pure culture have so far been unsuccessful. Fungal collections are maintained on living host plants under non-sterile conditions. Large-scale multiplication of efficient fungi has been achieved by inoculating appropriate host plants like clover, ray grass or sudan grass that are grown in disinfected soil or a variety of rooting media containing perlite, pumice, vermiculite, bark, sawdust, sand, gravel, peat or mixtures of these materials (Gianinazzi, 1982; Menge, 1984). Spore, hyphae, infected roots and infested soil or rooting medium obtained from this type of culture can, either separately or in mixture, constitute a source of crude inoculum.

Inoculant processing and application

The technique for processing microbial inoculants is very important. The successful use of these inoculants depends on a formulation that should be simple, economic, unaffected by long storage, and easy to transport and apply.

Peat-base inoculants The usual practice is to absorb the microbial culture on a protective carrier, generally peat (Williams, 1984). Alternative carriers include lignite coal, straw, cellulose, bagasses, ground crop residues, and soil. In some cases the microorganism is cultured and the inoculant is processed simultaneously. Ectomycorrhizal inoculants, for instance, are made of the fungal hyphae and the mineral substrate, usually vermiculite, imbibed with a nutrient medium on which the fungus is grown.

Recently, 'Les Tourbières Premier' (Quebec) have released into the North American market a peat-based substrate containing VA fungi (Mycori-mix Md) for pot cultures.

Soil-base inoculants (VA endomycorrhizae) In Dijon (France). Gianinazzi, Trouvelot & Gianinazzi-Pearson (1990*b*) have developed a method for producing soil-based inoculum containing $5 \cdot 10 \times 10^3$ infective propagules per kg, which can potentially yield up to $3 \times 10^3\ \mathrm{m}^{-3}$ inoculum per ha of glass house per year. A similar method has been developed by a sugar-beet company for producing inoculum outdoors in agriculture waste soil for commercial purposes (see *European Biotechnology Newsletter*, **61** (1989)).

Both types of inocula have given excellent results in nurseries producing woody ornamentals like Liquidambar, Lilac, Ampelopsis, Berberis in disinfected soils. Production was homogeneous and seedlings were marketable immediately after one season.

These soil-based inocula have also been successfully used, incorporated at a rate of 30%, in a compost or other artificial substrates for pot cultures. Where it is essential to reduce microbial contamination to a minimum, as for micropropagated plants, soil could be completely eliminated and surface-disinfected VA endomycorrhizal fragments used as inoculum. Methods ensuring rapid infection with a very low amount of inoculum (200 g for 500 plants) have given excellent results with different micropropagated plant species (Ravolanirina *et al.*, 1989; Gianinazzi *et al.*, 1990*b*). In Columbia, a soil-based inoculant is available in the market under the name *Manihotina*.

Inorganic clay carrier A more sophisticated, and therefore more expensive, form of VA endomycorrhizal inoculum has recently been put on the American market by Native Plants Inc. (USA). This inoculum, based on fungal spores, is incorporated into an inorganic (clay) carrier (Nutri-link ®) for use with potted plants or under field conditions (Wood, 1987).

Polymeric inoculants Another practice (Dommergues, Diem & Divies, 1979; Jung, Mugnier & Dommergues, 1982; Diem *et al.*, 1988; 1989) is based on the entrapment of the microbial cells in a polymer gel, generally as alginate beads. A recent improvement was achieved by adding clay (kaolinite) to the entrapping gel.

Polymeric inoculants containing either symbiotic microorganisms or PGPR, e.g. *Azospirillum*, have been successfully tested in numerous instances. To the best of our knowledge, large-scale experiments have only been carried out in a few cases.

A simple method for checking the quality of polymeric inoculants was recently proposed by Prin *et al.* (1989). This method is based on the evaluation of the dehydrogenase activity of the inoculant through an assay that uses the redox dye, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-(phenyltetrazolium chloride) (INT).

In practical terms, dried polymeric inoculants are best suited for use in nursery inoculations. After being dried they are applied to the seed bed or to the seeds after pseudo-solubilization by immersion in a buffer solution (Diem *et al.*, 1989).

Economics of biofertilizers

The benefit-cost analysis can be used to assess profitability of microbial inoculants. It is based on how much the present value of the benefit exceeds the present worth of the costs. When an enterprise has a benefit: cost ratio greater than 1 after the gross cost and gross benefit have been discounted at a suitable discount rate, most often the opportunity cost of capital, the enterprise is accepted as profitable. FAO (1984) gives some examples of benefit: cost ratios of legume inoculants. The price and cost of utilizing peat-base rhizobium inoculants is low, ranging from US \$0.24 per ha for white clover (*Trifolium repens*) to US \$6.46 per ha for *Vicia faba*. Inoculation costs for *Leucaena leucocephala*, *Cajanus cajan*, soybean (*Glycine max*) and *Vigna unguiculata* are between these two costs. Soil inoculation using peat granules at 10 kg per ha costs approximately US \$28.

FAO (1984) based the benefits from legume inoculants on N_2 fixed. They found benefit: cost ratios of 416 for white clover fixing 200 kg N_2 per ha and 17 for soybean fixing 100 kg N_2 per ha from inoculation, considering the cost of fixed N_2 in fertilizer N as US \$0.50 per kg. Valuation of benefits based on fixed N_2 is justified for leguminous cover crops, but for forage and grain legumes, the market prices are more meaningful to the farmer, or their shadow prices when social profitability is being evaluated. Economic analysis of using other biofertilizers should use crop dry matter production, forage and grain yield as the variables for benefit evaluation. Subba Rao (1986) used the price of total yield increases of various legumes in India to estimate the gain due to inoculation of selected legumes with rhizobium in 1978–9. Yield increases resulting from inoculation with rhizobium can also be expressed as the N fertilizer that would produce the same yield increases. The benefit is then the monetary value of this N fertilizer equivalent. Subba Rao (1986) also used this approach to estimate the pay-off to the farming community in India by the application of *Azospirillum* biofertilizer to various cereals and recorded savings of more than 2 million tonnes of urea equivalent to approximately US $0.67 × 10^9.

Yield variability and variable efficacy among agricultural fields is a major profit risk associated with the use of microbial inoculants and a significant obstacle to agronomic applications. Other risks are poor effectiveness of introduced microorganisms when conditions change, e.g. when pathogens acquire resistance to the inoculant, or when the inoculant strains mutate and lose effectiveness. For some biological control agents and PGPR, there may be a delay in effectiveness owing to the time necessary to produce antibiotics or phytohormones. Risk analyses have been developed to assist decision makers to select the best allocation of resources for maximum profit (Gittinger, 1984).

If benefit:cost ratios of microbial inoculant use are favourable, farmers still have to select microbial inoculants against a no-input system or against N or P fertilizers, herbicides, insecticides, fungicides and growth hormones. There would be no point in developing microbial inoculants that are less efficient or more expensive than a chemical treatment unless the chemical poses environmental or health problems. Partial budgeting analysis (PBA) can be used to compare profitability of various alternatives. The analysis is done by looking at the marginal cost, including opportunity cost, of adding a production activity and comparing it with the marginal increase in benefit that the new activity will bring (Gittinger, 1984). This budgeting approach does not include all production costs, but only those which change or vary between the farmer's alternatives including its current production practices. PBA is useful for decision making at each stage in the research–transfer–adoption process. But for an accurate analysis, all alternative technologies should be compared at their optimal levels, i.e. at their maximum profit. For rhizobium inoculants, for instance, a starter dose of N fertilizer as well as P and/or other nutrients may be needed. Yield response and production functions are rarely available in the literature. In the absence of data related to optimal levels of each technology, available data can be used as a first approximation of the relative profitability of the alternatives.

Because microbial inoculants are not expensive and have the potential to increase yields, their use must be economically profitable to smallholder farmers who have no access to agrochemicals. However, the technology will be adopted only if the input supply is adequate and reliable. Smallholder farmers use complex cropping systems to sustain yield, including the restorative bush fallow. Agronomic experiments should be designed to assess how microbial inoculants can improve these systems. For large-scale farmers, who practice monocropping or sequential cropping of sole crops, economic comparison of microbial inoculants and agrochemicals is straightforward. Considering the upward trend of the world-wide market opportunity for environmentally safe biopesticides (McIntyre & Press, 1989) and the present legume inoculant market, microbial inoculants can be considered in general as an economically profitable technology.

Rhizobium biofertilizer

The price of rhizobial biofertilizer expressed on 1 ha basis was reported to be US $1.7 in Egypt, US $5.2 in Zimbabwe, and US $4.5 in Rwanda. This price, which is the retail price for the farmers, is probably well under the actual cost of production in the government laboratories of these different countries.

Frankia inoculant

Using two methods of evaluation, M. Neyra (personal communication) calculated that the real cost of *Frankia* inoculant as it is prepared at BSSFT laboratory was in the range of 20–50 US cents per tree.

Mycorrhizal biofertilizer

According to F. Le Tacon (personal communication) the cost of ectomycorrhizal inoculation of trees would range from 10 to 50 US cents per tree. The cost of VA endomycorrhizae (Nutrilink) is 6 US cents per plant grown in 1–5 gallon containers and can be as low as $\frac{1}{4}$ US cent per plant when inoculation is performed in nursery beds (Nutrilink). Anyway the supplementary cost of using this inoculant is low when taking into account the beneficial effect on plant growth.

Blue-green algae (BGA)

BGA inoculation as recommended by Venkataraman (1981) is attractive because of its low cost. At 1981 prices, a yield increase of 30 kg per ha was sufficient to cover the cost of the inoculum (US $1.2 to 2.4). With an average increase in yield of 250 kg per ha, the benefit: cost ratio was about 1 to 10. The low investment and the high expected relative return probably explain why BGA inoculation was recommended before the method was fully proved. But BGA inoculation is far from being consistently successful as reported in a study of the economics of BGA use by 40 farmers in Tamil Nadu which shows a non-significant $4 per ha return for BGA utilization (Roger, 1990).

Crop vs tree biofertilizers

When calculating the cost of biofertilizers on a per hectare basis for crop vs tree biofertilizers, it is clear that tree inoculation is much cheaper than crop inoculation since the density of forest plantation is *c.* 2000 plants per ha whereas that of crops is in the range of 200 000 to 500 000 plants per ha. Consequently even if the cost of biofertilizers for trees is higher than that for crops (because of the wider range of strains to be used), the benefit: cost ratio will always be much higher than 1.

Conclusion

Except for rhizobial inoculation the use of biofertilizers in tropical agriculture is still largely under-utilized. In forestry, with the exception of ectomycorrhizae, the use of biofertilizers is even more limited.

This is surprising since good and reliable biofertilizers are available now, e.g. *Frankia*, or VAM endomycorrhizal fungi. Their use is restricted since they are not yet commercially available.

Another reason for the limited use of biofertilizers could be in the misconception that their cost is too high. In fact their price is much lower than that of conventional agrochemicals. In the specific case of trees inoculation with the compatible symbionts, biofertilizers are still much more cost effective than in the case of annual crops.

The poor expansion of biofertilizers could also be explained by the fact that the methods for the carriers to develop longer shelf life and survival are not reliable; however, recent advances have been made which ensure much better survival of the microorganisms and easy transportation and application. More investigations should be made in the future to improve the target strains through usual selection practices and genetic engineering. Also more attention should be given to the impact of physical, chemical and biological limiting factors occurring in the field. In other words, good culture techniques and adequate irrigation, should be implemented to improve the efficiency of biofertilizers, thus increasing their benefit: cost ratio.

Finally to reduce the costs, biofertilizers should be applied only in situations that are predictably conductive to a worthwhile response.

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