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INOCULANTS OF PLANT GROWTH-PROMOTING BACTERIA FOR USE IN AGRICULTURE

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ABSTRACT

An assessment of the current state of bacterial inoculants for contemporary agriculture in developed and developing countries is critically evaluated from the point of view of their actual status and future use. Special emphasis is given to two new concepts of inoculation, as yet unavailable commercially: (i) synthetic inoculants under development for plant-growth promoting bacteria (PGPB) (Bashan and Holguin, 1998), and (ii) inoculation by groups of associated bacteria.

This review contains: A brief historical overview of bacterial inoculants; the rationale for plant inoculation with emphasis on developing countries and semiarid agriculture, and the concept and application of mixed inoculant; discussion of microbial formulation including optimization of carrier-compound characteristics, types of existing carriers for inoculants, traditional formulations, future trends in formulations using unconventional materials, encapsulated synthetic formulations, macro and micro formulations of alginate, encapsulation of beneficial bacteria using other materials, regulation and contamination of commercial inoculants, and examples of modem commercial bacterial inoculants; and a consideration of time constraints and application methods for bacterial inoculants, commercial production, marketing, and the prospects of inoculants in modern agriculture.

INTRODUCTION

Brief Historical Background

Inoculation of plants with beneficial bacteria can be traced back for centuries. From experience, farmers knew that when they mixed soil taken from a previous legume crop with soil in which

nonlegumes were to be grown, yields often improved. By the end of the 19th century, the practice of mixing "naturally inoculated" soil with seeds became a recommended method of legume inoculation in the USA (Smith, 1992). A decade later, the first patent ("Nitragin") was registered for plant inoculation with *Rhizobium* sp. (Nobbe and Hiltner, 1896). Eventually, the practice of legume inoculation with rhizobia became common.

For almost 100 years, *Rhizobium* inoculants have been produced around the world, primarily by small companies. Some legumes, like the soybean *(Glycine max* (Merr.)L.) in Brazil, are not fertilized with nitrogen, but are only inoculated (Döbereiner, lecture in: VI *Azospirillum* conference, Sárvár, Hungary, 1994). Apart from soybean inoculation, which has made a major agricultural impact in the USA, Brazil, and Argentina, significant contributions to the production of other legumes were made in Australia, North America, Eastern Europe, Egypt, Israel, South Africa, New Zealand, and, to a lesser extent, Southeast Asia. For the large majority of less developed countries in Asia, Africa, and Central and South America, inoculant technology has had no impact on productivity of the family farm, because inoculants are not used or are of poor quality (Eaglesham, 1988).

Inoculation with nonsymbiontic, associative rhizosphere bacteria, like *Azotobacter*, was used on a large scale in Russia in the 1930s and 1940s. The practice had inconclusive results and was later abandoned (Rubenchik, 1963). Interest in *Azotobacter* as an inoculant for agriculture has only recently been revived. An attempt to use *Bacillus megaterium* for phosphate solubilization in the 1930s on large scale in Eastern Europe apparently failed (Macdonald, 1989).

Two major breakthroughs in plant inoculation technology occurred in the late 1970s: (i) *Azospirillum* was found to enhance nonlegume plant growth (Döbereiner and Day, 1976), by directly affecting plant metabolism (Bashan and Holguin, 1997a: 1997b), and (ii) biocontrol agents, mainly of the *Pseudomonas fluorescens* and *P. putida* groups, began to be intensively investigated (Défago et al. 1992; Kloepper and Schroth, 1981; Glick, 1995: Glick and Bashan, 1997). In recent, years, various other bacterial genera, such as *Bacillus, Flavobacterium, Acetobacter*, and several *Azospirillum*- related microorganisms have also been evaluated (Kloepper, 1994; Tang, 1994; Tang and Yang, 1997).

The immediate response to soil inoculation with associative, nonsymbiotic PGPB (but also for rhizobia) varies considerably depending on the bacteria, plant species, soil type, inoculant density and environmental conditions. In general, shortly after the bacteria are introduced into the soil, the bacterial population declines progressively (Bashan and Levanony, 1988; van Elsas et al. 1986). This phenomenon (together with bacterial biomass production and the physiological state of the bacteria in the inoculant, discussed below) may prevent the buildup of a sufficiently large PGPB population in the rhizosphere to obtain the intended plant response. The key obstacle is that soil is a heterogeneous and unpredictable environment, even on a small scale (van Elsas and van Overbeek, 1993). The inoculated bacteria sometimes

cannot find an empty niche in the soil for survival except in sterilized soil, a condition which does not exist in large-scale agriculture. They must compete with the often better-adapted native microflora and withstand predation by protozoans. A major role of inoculant formulation is to provide a more suitable microenvironment (even temporarily) to prevent the rapid decline of introduced bacteria in the soil. Although much is known about the survival of bacteria within the protective environment of an inoculant carrier, little is known about the stresses that bacteria must endure upon transfer to the competitive and often harsh soil environment (Heijnen et al. 1992; Rodriguez-Navarro et al. 1991; van Elsas and Heijnen, 1990). Inoculants have to be designed to provide a dependable source of beneficial bacteria that survive in the soil and become available to the plant.

Although *Rhizobium* inoculants have been in the marketplace for a century, it is only recently that the first commercial preparations of PGPB have appeared on the market (Fages, 1992, Tang, 1994; Tang and Yang, 1997). Kenney (1997) noted that "Biological products have had a less than spectacular penetration of the chemical pesticide market. Although great promises have been made, the fulfillment of those promises has not met expectations".

The aims of this review, apart from providing a comprehensive compendium of scattered, hard-to-find, practical information are: (i) to point out present and possible future trends in inoculant technology, and (ii) to mark possible future research pathways for the construction of better inoculants. No attempts have been made to collect technical or marketing data on the numerous inoculants currently available in the market.

Terms and Definitions

"Bacterial inoculant" - A formulation containing one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material, either organic, inorganic, or synthesized from defined molecules. The inoculant is the means of bacterial transport from the factory to the living plant. The desired effects of the inoculant on plant growth can include nitrogen fixation in legumes, biocontrol of (mainly) soil-borne diseases, the enhancement of mineral uptake, weathering of soil minerals, and nutritional or hormonal effects. Bacterial inoculants may require lengthy and expensive registration procedures in some countries.

"Biofertilizer" - A misleading but widely used term meaning "bacterial inoculant". Usually it refers to preparations of microorganism(s) that may be a partial or complete substitute for chemical fertilization (like rhizobial inoculants). However, other bacterial effects on plant growth are largely ignored. The reason for using the word "fertilizer" is that in some countries it allows easier registration for commercial use. This term, although is appropriate for rhizobia, should be abandoned.

THE SCIENTIFIC RATIONALE FOR PLANT INOCULATION WITH EMPHASIS ON DEVELOPING COUNTRIES AND SEMIARID AGRICULTURE PRACTICES

The first objective when considering inoculation with beneficial bacteria is to find the best bacteria available (Bashan and Holguin, 1997; Bashan et al. 1993; Glick, 1995; Kloepper et al. 1989). Next, a study of the specific inoculant formulation is generally undertaken. In practical terms, the chosen formulation determines the potential success of the inoculant (Fages, 1992). Many potentially useful bacteria reported in the scientific literature never appear on the commercial market, perhaps because of inappropriate formulation.

The technical optimization of an inoculant formulation is independent of the strain used because most of the strains of the same bacterial species share many physiological properties. It may be assumed that a technological process developed for a particular strain is readily adaptable to another strain of the same species with only minor modifications. This normally does not apply to different bacterial genera (Anonymous, 1995; Fages, 1990, 1992). Apart from this "rule of thumb", strain-formulation combinations should be optimized in preparations causing stress for the bacteria like dry powders or liquids (discussed later, Anonymous, 1996). Formulation options are usually considered at the inoculant producer's Research and Development facility, located primarily in developed countries where the target market exists. Often, the unique problems of applying these inoculants in developing countries are not considered, since these countries represent only a small share of the market. To highlight this point, I will briefly summarize the main difficulties facing the application of microbial inoculants in some developing countries.

Agricultural practices in developing countries or under semiarid conditions are two examples in which bacterial inoculants may find their greatest challenges (Anonymous, 1995).

Developing countries practice mainly low-input agriculture in which fertilizers, pesticides, and agrotechnical machinery are scarce. The financial resources of the individual farmer in a family' farm system are small and the availability of bank loans is extremely limited. For example, about 80% of Mexico's farm and grazing land is owned, rented, or worked by poor families. Many farmers are only part-time agricultural laborers who receive a daily wage or a percentage of the crop (Anaya-Garduño, 1994). Yields of legumes grown in family farms in developing countries are often meager and fall far short of those that can be obtained under experimental conditions. It is irrelevant whether or not responses to bacterial inoculation can be demonstrated under fertile, high-yield conditions in experimental stations in these countries or in developed countries, since this has little relevance to subsistence farming (Summerfield and Lawn, 1987). Naturally, this type of farming does not have the resources to invest in improved agricultural techniques. Artificial inoculation, in particular, requires an infrastructure to store and transport biological products in large quantities into rural areas, and this infrastructure is not available. In

developing countries, even the wealthier growers lack sufficient knowledge of modern agricultural techniques. Governmental extension services (there are almost no private consultancies) and the growers' formal agricultural education are poor. Most growers tend to practice traditional methods or copy methods from more developed countries without being aware of the deficiencies of such practices in their own particular region or without knowing the "cost" to the environment. In most cases, fertilizers are too expensive or the crop's value does not justify the expense (Anonymous, 1995). In places where fertilizers are available, overfertilization is common (Bashan et al. 1992), and this practice may contaminate deep water reservoirs, produce significant health hazards to the nearby population, and disrupt the local environment (Anonymous, 1995; Turrent-Fernández, 1994). In several Latin American, African, and Pacific Island countries, a shortage of foreign currency to purchase large quantities of imported fertilizers is the main limiting factor. Consequently, most yields are low and much of the agricultural production is destined for self-consumption or local markets (Craswell and Morris on, 1994). The contribution of such agriculture to the world's food supply remains well below its agronomic potential (Turrent-Fernández, 1994). The use of beneficial microorganisms in these areas is almost unheard of, except for rhizobia, which were intro duced by many governments in the past. Many growers, when asked to replace available nitrogen fertilizer with rhizobial inoculants, are reluctant to do so for fear of reducing soil fertility (R.S. Smith, 1995, personal communication). Except for soybeans, inoculants have had very little impact on legume production in developing countries. Furthermore, in many developing countries, there are no inoculant industries (as in all of Central America) or usable peat sources (as in India) for traditional inoculant formulations (described later) (Eaglesham, 1988), which increases the difficulty of popularizing the inoculation concept. Finally, in many developing countries, there is a basic reluctance to use bacteria and fungi as beneficial organisms, because in these rural cultures, microbes, are perceived to be associated with human and animal diseases (Anonymous, 1995).

Semiarid conditions, make survival difficult for the introduced bacteria even in developed countries like Israel, Australia, and parts of the USA. Harsh conditions, including frequent droughts, lack of sufficient irrigation, high salinity and soil erosion, may quickly diminish the population of any bacteria introduced into the soil unless precautions are taken to select the proper inoculant and provide irrigation concomitant with inoculation. Inoculation should be timed to coincide with sowing into moist soil or be delivered quickly with irrigation to assure rapid colonization of the target plants, as practiced in developed countries. Semiarid agriculture in developing countries, however, is a greater challenge (Anonymous, 1995), and technologically speaking, it seems destined for failure. However, in this type of agriculture, beneficial microorganisms may make the greatest contribution, if inexpensive and easy-to-use formulations can be developed. There, the inoculated bacteria would be subject to few, if any, side effects

from pesticides. If properly instructed, the farmer, by virtue of cultivating small family plots, can apply inoculants at the right time and at the right dosage. Since the yields are low in the first place, any enhancement will be greater than in developed agriculture (Hegazi,1988) in which plants are already grown almost at their maximum genetic potential. Finally, most of the world's farmers are located in developing countries, although their productivity is presently only a small fraction of that of developed countries. Since microbial inoculants can be produced and marketed inexpensively (McIntyre and Press, 1991), with some organized financial aid and better information transfer, these farmers are potential clients for microbial technology (Anonymous, 1995).

In summary, microbial inoculants can play an increasing role in the agriculture of developing countries, but field procedures involving their use should first be conceived in developed countries where the capacity for R&D is greater (Glick et al. 1991).

MIXED BACTERIAL INOCULANTS: CONCEPT AND POTENTIAL FOR THE FUTURE

Numerous recent studies show a promising trend in the field of inoculation technology. Mixed inoculants (combinations of microorganisms) that interact synergistically are currently being devised. Microbial studies performed without plants indicate that some mixtures allow the bacteria to interact with each other synergistically, providing nutrients, removing inhibitory products, and stimulating each other through physical or biochemical activities that may enhance some beneficial aspects of their physiology, like nitrogen fixation. It still has to be demonstrated that these bacterial synergistic effects also benefit plant growth. An example of this is *Azospirillum*, one of the most studied bacteria that associates with plants (Bashan and Holguin, 1997a). It may associate with sugar- or polysaccharide-degrading bacteria (PDB), establishing a metabolic association where the sugar-degrading bacteria produce degradation and fermentation products used by *Azospirillum* as a carbon source, which in turn provides PDB with nitrogen. Other examples are the association between *Azospirillum* and *Bacillus* that degrades pectin, *Azospirillum* and *Cellulomonas* that degrades cellulose, and *Azospirillum* and Emerobacter cloacae that ferments glucose (Kaiser, 1995; Khammas and Kaiser, 1992; Halsall, 1993).

Plant studies have shown that the beneficial effects of *Azospirillum* on plants can be enhanced by co-inoculation with other microorganisms. Co-inoculation, frequently, increased growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients (For recent reviews see Bashan and Holguin, 1997a, b). Thus, plant growth can be increased by dual inoculation with *Azospirillum* and phosphate-solubilizing bacteria (Alagawadi and Gaur, 1992; Belimov et al., 1995). *Azospirillum* is also considered to be a *Rhizobium*-"helper" stimulating nodulation, nodule activity, and plant metabolism, all of which stimulate many plant growth

variables and plant resistance to unfavorable conditions (Andreeva et al., 1993; Fabbri and Del Gallo, 1995; Itzigsohn et al., 1993). Other successful combinations include *Azospirillum* or *Azotobacter* mixed with Streplomyces (Elshanshoury, 1995), and *Azospirillum* with the fungal biocontrol agent, Phialophora radicola (Flouri et al., 1995).

Mixed inoculation with diazotrophic bacteria and arbuscular-mycorrhizal fungi creates synergistic interactions that may result in a significant increase in growth, in the phosphorus content in plants, enhanced mycorrhizal infection, and an enhancement in the uptake of mineral nutrients such as phosphorus, nitrogen, zinc, copper, and iron (Al-Nahidh and Gomah, 1991; Barea, 1997; Chanway and Holl, 1991; Garbaye, 1994; Gori and Favilli, 1995; Isopi et al. 1995; Li and Hung, 1987; Li et al. 1992; Linderman, 1992; Linderman and Paulitz, 1990; Rozycki et al. 1994; Singh et al., 1990).

These studies point out further advantages of mixed cultures over single strains: i) in vitro studies have shown that *Azospirillum* can produce more phytohormones when grown in mixed culture (Janzen et al., 1992), ii) mixed cultures provide conditions more suitable for nitrogen fixation than pure cultures (Drozdowicz and Ferreira-Santos, 1987; Holguin and Bashan, 1996; Lippi et al., 1992), and iii) mixed inoculation of biocontrol microorganisms is more efficient in controlling pathogens than the use of single-strain inoculants, e.g., combinations of *Pseudomonas* with *Serratia* (Frommel et al., 1991) and *Pseudomonas* with a nonpathogenic *Fusarium* (Lemanceau and Alabouvette, 1991).

Despite progress in research on mixed inoculants, they are not yet produced commercially. In Canada, for example, apart from rhizobial inoculants, there are only two registered (single) microbial commercial inoculants Polonenko (1994), and more single strains of microbial inoculants must be registered before the inoculation industry can contemplate the development and commercialization of multibacterial inoculants.

INOCULANT FORMULATIONS

Optimal Charasterics of a Carrier For Inoculants

The carrier is the delivery vehicle of live microorganisms from the factory to the field; however, no universal carrier or formulation is presently available for the release of microorganisms into soil (Trevors et al. 1992). The carrier is the major portion (by volume or weight) of the inoculant. The materials of which the carrier is composed and the type of formulation vary. The carrier can be a slurry or a powder. A good carrier should have one essential characteristic: the capacity to deliver the right number of *viable* cells in good physiological condition at the right time (Bashan, 1986c, 1991; Fages, 1990, 1992; Smith, 1992; Trevors et al. 1992). Additional desirable characteristics for a good inoculant should be as follows:

(i) Chemical and physical characteristics. The inoculants should be nearly sterile or easily sterilized, and as chemically and physically uniform as possible. They should also be of consistent quality, high water-holding capacity (for wet carriers) and suitable for as many bacterial species and strains as possible.
(ii) Manufacturing qualities. The inoculant should be easily manufactured and mixed by existing industry, it should allow for the addition of nutrients, have an easily adjustable pH, and be made of a reasonably priced raw material in adequate supply.
(iii) Farm handling qualities. A good inoculant allows for ease of handling (a major concern for the farmer), provides rapid and controlled release of bacteria into the soil, and can be applied with standard agrotechnical machinery.
(iv) Environmental characteristics. The inoculant should be nontoxic, biodegradable and nonpolluting, and should minimize environmental risks such as the dispersal of cells to the atmosphere or to the ground water.
(v) Storage qualities. The inoculant should have sufficient shelf life (one or two years at room temperature is often necessary for successful integration into the agricultural distribution system in some countries)

Naturally, no single carrier can have all these qualities, but a good one should have as many as possible. A "super-inoculant" such as the one described above is theoretically possible. To date, no effort to synthesize a carrier with predefined superior characteristics has been reported, probably because of the cost involved (anonymous, 1995). The raw materials of most commercial carriers are cheap and naturally abundant (peat and soil fractions).

Types of Existing Carriers For Inoculants

The almost universal carrier for rhizobia (which is the only inoculant being sold in large volume today) is peat (Bezdicek, 1978; Burton, 1967; Smith, 1987; Williams, 1984). Despite its popularity, it has several drawbacks (discussed below), and many alternative materials have been evaluated.

Carriers can be divided into four basic categories:
(i) Soils: peat, coal, clays, and inorganic soil (Chao and Alexander, 1984; Kotb and Angle, 1986; Packowski and Berryhill, 1979; Smith, 1995; Singh and Sharma, 1973).
(ii). Plant waste materials: composts, farmyard manure, soybean meal (Iswaran et al., 1972), soybean and peanut oil (Kremer and Peterson, 1982a,b), wheat bran (Jackson et al. 1991), "press-mud" (a by-product from the sugar industry, Philip and Juhari, 1984), agricultural waste material (Sadasivam et al. 1986), spent mushroom compost (Bahl and Juhari, 1986), and plant debris (Richter et al. 1989).

(iii) Inert malerials: vermiculite (Paau et al. 1991; Sparrow and Ham, 1983 a,b), perlite, ground rock phosphate, calcium sulfate, Polyacrylamide gels (Dommergues et al. 1979), and alginate beads (Aino et al, 1997; Bashan, 1986a; Jung et al. 1982; Sougoufara et al. 1989).
(iv) Plain lyophilized microbial cultures (Mohammadi, 1994 a,b) and oil-dried bacteria (Johnston, 1962). These preparations can later be incorporated into a solid carrier or used as they are.

To produce an inoculant, the target microorganism can be introduced into a sterile or non sterile carrier. From a purely microbiological point of view, the sterile carrier has significant advantages but has not usually been cost effective from a commercial standpoint. Nonetheless, sterile-originated inoculants have been successfully marketed even with their higher price tag (Anonymous, 1996). Table 1, which summarizes the advantages and disadvantages of each carrier type, shows that both types of carriers have their niche in the inoculant market. However, the cheaper nonsterile carriers, despite their potential disadvantages, have a much larger slice of the market (Olsen et al. 1994a,b).

Formulation is *the* crucial issue for inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. Formulation is the industrial "art" of converting a promising laboratory-proven bacterium into a commercial field product. Chemical formulations of agroproducts set high standards for long shelf life, ease of use, and resistance to abuse by the farmers. Microbial inoculant formulations are expected to match the above characteristics and overcome two major problems for living organisms: (i) loss of viability during short storage in the grower's warehouse (which in developing countries usually lack refrigeration), and (ii) long shelf life and stability over the range of -5º to 30ºC within the marketing distribution systems. Products lacking this range of temperature tolerance will be unacceptable in the agricultural market (Kenney, 1997; Lethbridge, 1988).

Inoculants come in four basic dispersal forms:
(i) Powders. This form is used as a seed coating before planting. The smaller the particle size, the better the inoculant will adhere to the seeds. Standard sizes vary from 0.075 to 0.25 mm, and the amount of inoculant used is around 200 to 300 g/ha. These inoculants are the most common both in developed (Smith, 1997) and developing countries (Tang and Yang, 1997).
(ii) Slurries. This inoculant is based on powder-type inoculants suspended in liquid (usually water). The suspension is directly applied to the furrow or alternatively, the seeds are dipped just prior to sowing.
(iii) Granulars. These inoculants are applied directly to the furrow together with the seeds. Size ranges are from 0.35 to 1.18 mm. Rhizobia inoculant is used at a rate of 5 to 30 Kg/ha. These inoculants are popular and have been successfully commercialized since 1975 (Anonymous, 1996; Tang, 1994; Tang and Yang, 1997). Bead-like forms are synthetic variations of granular forms. These can be in macro sizes (1 to 3 mm in diameter) used as granules form, or in micro size (100 to 200 ìm) used as a powder for seed coating. These inoculants are a new, as yet unproven, possibility in inoculation

Table 1. Comparison of sterile and nonsterile peat-based inoculants

Inoculant parameters	**Sterile**	**Non-sterile**
Population of beneficial bacteria	High	Variable
Longevity	High, but depends on the carrier material	Relatively low
Addition of nutrients to the carrier to increase final population	Possible without sacrificing the final quality of the inoculant	Not always possible because many contaminants grow faster than many beneficial bacteria
Choice of materials to be used as carriers	Many materials are not easily sterilized or change their chemical and physical composition upon sterilization	Almost unlimited
Labor requirements	Skilled and expensive	Mostly unskilled and less expensive
Sterilization equipment required	Large autoclaves are costly to purchase and operate. Sterilization by irradiation, not always available	Not needed
Sterile production space	Large and costl	Not needed
Monitoring of contamination	Essential for quality control of the product	Essential for quality control of the product
Total cost of production	High	Much lower than sterile preparations

technology, and their features will be described later in detail.

(iv) Liquids. These inoculants use broth cultures or liquid formulations, mainly in water, but also in mineral or organic oils. The seeds are either dipped into the inoculant before sowing, or an applicator evenly sprays the liquid inoculant on the seeds. After drying, the seeds are sown. This method ensures even coverage of the seeds without interference with the seed monitoring system of the planters or inoculum loss when dried (Smith, 1995). These inoculants are currently popular in the USA, Canada, Argentina, and Brazil, mainly for soybeans, but also for lentils, peas, and peanuts (R.S. Smith, 1995, personal communication). For biocontrol agents of leaf diseases, the inoculant can be diluted in water and sprayed for better coverage of the leaves (Daayf et al. 1995). Alternatively, the suspension can be sprayed directly into the furrow or on the seeds before sowing. The in-furrow inoculant provides a larger amount of bacteria to the plant than seed inoculation. In rhizobia, this improves plant nodulation (Smith, 1995). For bacteria with poor survival in the soil, like *Azospirillum* sp. (Bashan et al. 1995), these formulations are largely useless since they do not provide a protective environment for the bacteria. Furthermore, in some plant species, these formulations should be applied several days after sowing at seedling germination, causing extra work and cost for the farmer.

The microbial inoculant is not merely a suitable carrier containing the bacteria. Other materials might be involved in the final formulation. For example: an *Azospirillum lipoferum* inoculant for corn, developed in France, was based on 1% alginate containing the bacterial cells and 99% inert calcium carbonate "diluent", which allowed for the right bacterial concentration, because the alginate contained too many cells of *Azospirillum* for optimal inoculation (Anonymous, 1995). Apparently this alginate formulation was never commercialized, perhaps due to its high cost, and *A. lipoferum* was commercialized in a sterile peat inoculant instead (Anonymous, 1996).

The use of each type of inoculant depends upon market availability, cost, and the needs of a particular crop under specific environmental conditions. For example, the granular form is better than powder inoculants for rhizobia, under stressful planting conditions, but since more is required, it is costlier (Smith 1992).

Traditional Peat Formulations

Peat formulations have been the carriers of choice, and are the most commonly used in the rhizobia inoculation industry. Since it was adopted decades ago, farmers are by now quite comfortable with peat, and governmental agencies usually know how to monitor its quality. Its popularity is primarily due to successful field results obtained under commercial cultivation. In this form, the bacteria are metabolically active, and in some inoculants, bacterial multiplication continues during the storage period, as long

as sufficient nutrients, moisture, and the correct temperature are maintained.

Recipes for peat inoculants are widely known (Burton, 1967,1976; Smith, 1992; Thompson, 1980) and will not be repeated here. Instead, the major drawbacks of such inoculants and why other types of carriers are being sought are discussed. The principle drawbacks originate from the great variability in peat quality (which is source dependent), and because peat is an undefined, complex organic material. This greatly affects the final product and may cause difficulties in inoculant dosage, storage conditions (van Elsas and Heijnen, 1990), and variation of inoculant effectiveness between different manufacturers and between different batches from the same manufacturer (Bashan, 1992, unpublished data). Heat sterilization of some peats may release compounds toxic to the bacteria resulting in low bacterial counts (Chao and Alexander, 1984). In peat inoculants, bacteria have a lower tolerance for physical stress during storage, in particular for temperature variations. Some types of peat can even reduce plant growth (Huber et al. 1989). Finally, peat formulations are prone to contamination that can reduce the shelf life of the inoculant (Fages 1992; Olsen et al. 1994a,b; van Elsas and Heijnen, 1990). From the delivery standpoint, peat powder is easily blown away from the seeds by the commonly used seed air-delivery system used by the planter. Peat also interferes with the seed monitoring mechanism of the planters. Addition of adhesives to the inoculant during its application to the seeds or slurry application will improve its adhesion, but that requires additional time and labor for a process that is already labor-intensive (Smith, 1995).

New Trends in Formulations Using Unconventional Synthetic Materials

During the last decade, several experimental formulations based on polymers have been evaluated. These polymers have demonstrated potential as bacterial carriers (Jung et al. 1982) that offered substantial advantages over peat. These formulations encapsulate the living cells (described below), protect the microorganisms against many environmental stresses, and release them to the soil, gradually but in large quantities, when the polymers are degraded by soil microorganisms, usually at the time of seed germination and seedling emergence. They can be stored dried at ambient temperatures for prolonged periods, offer a consistent batch quality and a better defined environment for the bacteria, and can be manipulated easily according to the needs of specific bacteria. These inoculants can be amended with nutrients to improve the short-term survival of the bacteria upon inoculation, which is essential to the success of the inoculation process, especially with associative PGPB. However, *a major constraint* for the inoculation industry is that polymers are expensive compared to peat-based inoculants and require more handling by the industry (Fages, 1992). Thus, even relatively large inoculant manufacturers have currently abandoned the technique (R.S. Smith, 1995).

Encapsulated Formulations

The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial-inoculation technology. At present there is no commercial bacterial product using this technology.

The concept underlying immobilized microbial cells, is to entrap beneficial microorganisms into a matrix. The formulation (bacteria-matrix) is then fermented in a bacterial growth medium. These formulations can produce many useful compounds for industrial and environmental applications (such as organic acids, amino acids, enzymes) and biodegrade toxic materials (bioremedation) over extended periods of time. The bacterial products are then extracted from the bioreactor while fermentation continues (Bettman and Rehm, 1984; Chibata and Tosa, 1977; Lin and Wang, 1991). An excellent review of environmental applications of immobilized microbial cells was recently published (Cassidy et al. 1996).

Immobilized microbial cells are easy to produce, store, and handle during industrial operations. The main goal of these industrial formulations is to maintain the cells entrapped in an active form for as long as possible. Any premature release of the microorganisms from these encapsulated forms is undesirable. Encapsulated bacterial formulations in agriculture have at least two distinctly different goals from those of the fermentation industry: (i) to temporarily protect the encapsulated microorganisms from the soil environment and microbial competition, and (ii) to release them gradually for the colonization of plant roots (Bashan, 1986a; Bashan and Carrillo, 1996; Digat, 1991).

Macro and Micro Formulations of Alginate

Alginate is the material most commonly used for encapsulation of microorganisms. The resulting inocula are used for various purposes: the immobilization of cell organelles and enzymes, the application of biological control agents and mycoherbicides, to increase the stability of recombinant plasmids in the host cells, in bacterial chemotaxis research, and mushroom cultivation (Bashan and Holguin, 1994; De Taxis du Poet et al. 1986; Kierstan and Bucke, 1977; Romaine and Schlagnhaufer, 1992; Walker and Connick, 1983). Alginate is a naturally occurring polymer composed of f1-1,4-linked D-mannuronic acid and L-glucuronic acid. It is available from different macroalgae (DeLucca et al. 1990) as well as several bacteria (Smidsrod and Skjak-Braek, 1990). Because of the massive production of alginate in the far east, alginate cost has recently dropped (Anonymous, 1995) making it potentially more attractive to the inoculant industry.

The preparation of beads containing bacteria is fairly easy and involves a multistep procedure (Bashan, 1986a; Digat, 1991). In cases where the biomass of the entrapped strain is low, a secondary multiplication of the entrapped bacteria in the already formed beads is required (Bashan, 1986a).

Several other alginate-based preparations have been tried for the encapsulation of VAM fungi (Gamy et al. 1982), ectomycorrhizal fungi (Marx and Kenney, 1982; Le Tacon et al. 1985), *Frankia* inoculation (Sougofara, 1989), and fungi used as biocontrol agents against soil-borne pathogens (Fravel et al. 1985; Lewis and Papavizas, 1985).

The main advantages of alginate preparations are their nontoxic nature, biodegradability, and their slow release of microorganisms into the soil (Bashan, I 986a; Kitamikado et al. 1990). This technology was used to encapsulate the plant-beneficial bacteria *A. brasilense* and *P. fluorescens* (Bashan, 1986a), which were later successfully used to inoculate wheat plants under field conditions. The bacteria survived in the field long enough and their populations were comparable to the survival of bacteria originating from peat-based inoculants (Bashan et al. 1987). The encapsulation of genetically engineered *P. fluorescens,* which was released into the soil, showed significantly improved survival rates over nonencapsulated cells after 3 months. Furthermore, the addition of clay and skim milk to the beads significantly increased bacterial survival over alginate beads alone. Colonization of wheat roots by beneficial cells released from the beads was superior to that achieved by direct soil inoculation. These studies provide clear evidence that alginate beads are efficient slow-release carriers for plant inoculants, providing a protective environment in the soil. *P. fluorescens* numbers decreased only moderately in the soil when the cells were encapsulated, whereas a large reduction in bacterial numbers was observed when free, nonencapsulated inoculum was used. When the bacteria were encapsulated, they were washed down only slightly compared to. free inoculated bacteria. Most of them remained in the root zone (van Elsas et al. 1991, 1992). Another recent example is the colonization of tomato roots with *P. fluorescens* embedded in alginate beads to produce a biocontrol preparation against bacterial wilt of tomato (Aino et al, 1997). Trevors et al. (1992) proposed that good rhizosphere colonization and survival, a low wash-down rate, and resistance to drying show that the use of alginate in bacterial inoculation, has potential.

Alginate preparations may have solved many of the problems associated with traditional peat inoculants. A comparison between the features of basic alginate formulations and peat formulations is presented in Table 2.

The use of macro alginate beads has two major disadvantages: (i) the need for an additional treatment during sowing, and (ii) the need for the bacteria to move through the soil towards the plants (Bashan and Holguin, 1995; Bashan and Levanony, 1987). In developed countries, a farmer who is already busy during sowing might be pressed for time and reluctant to incur the additional expense (Anonymous, 1995). In developing countries, the farmer might not inoculate the seeds at all. This is because of insufficient agricultural education and conservative traditions that make them suspicious of new technologies, especially those involving live bacteria (Anonymous, 1995). Under agricultural practices, when beads are loosely mixed with seeds and sown together, the beads containing the bacteria might fall far from the seeds (up to several cm).

Table 2. Comparisons of alginate inoculant and peat inoculant

Alginate	Peat
No inexpensive industrial technology exists	Various successful industrial processes exist
Chemically and physically uniform	Non-uniform (complex organic matter
Biodegradable in the soil	Biodegradable in the soil
Simple to use for the farmer	Simple to use for the farmer
Inexpensive raw material	Inexpensive raw material
Can be produced easily b industry	Is produced easily b industry
Produces no environmental pollution, non-toxic and biodegradable	Produces no environmental pollution slightly increasing the organic matter of the soil, non-toxic and biodegradable
More consistent root colonization with PGPB (Experimental only)	Erratic root colonization with some PGPB, more consistent with rhizobia even under commercial usage
Bead strength and release of bacteria is controlled and can be easily manipulated during formulation	Release of bacteria can not be controlled
Quality control is technically simple	Difficult to maintain the same quality between batches
Long shelf-life at ambient temperature	Limited shelf life even at 5° C (about 1 year for non-sterile and 2 ears for sterile
Storage requires little space	Bulk
Can not be contaminated after production	Easily contaminated under inappropriate storage and contains all kinds of contaminants especially in common non-sterile preparations
Long term survival in soil under water field capacity	Survival of rhizobia is just long enough to incur root colonization
Resistant to moisture fluctuations	Susceptible to moisture fluctuations
No direct effect of the alginate polymer on plant growth	Some peals inhibit plant growth
Possible to add nutrients for auxotrophic bacteria or to accomodate special nutritional requirements of some bacteria. Nutrition also increases survival time.	Nutritional supplements in sterile preparations only
Can be loaded with a to 10^{11} cfu/ inoculant	Inoculants normally do not exceed 10^{8} cfu/
Bacteria in beads are dry until rain comes. Seed germination after rain is accompanied by "awakening " bacteria.	Needs wet conditions immediately after application

The bacteria released from the beads must migrate through the soil, facing competition from the native microflora and sometimes the absence of a continuous film of water needed for their movement. These distances might prove prohibitive for many beneficial bacteria, even those with a proven motility in soil like *Azospirillum* (Bashan, 1986c, Bashan and Levanony, 1987).

To overcome these difficulties, the microbead concept was conceived. If the beads are small enough but still capable of encapsulating a sufficient number of bacteria, it would be possible to produce a powder-like formulation. The seeds would be coated with this "bead dust" at the seed handling facility and sold to the farmer in developing countries as "improved seeds". Coated seeds (but with fertilizers or fungicides) are common and accepted in most rural areas of Mexico, for example. In developed countries, in large scale agricultural practice such as in North America, precoated seeds will eliminate the need for an additional expensive field treatment and provide the ultimate convenience for the grower. To be sure, precoating seeds with bacteria is not an easy industrial task (Paul et al. 1991), however, a similar idea, but with peat inoculant, has already been applied for preinoculation of forage legumes such as alfalfa. The peat is applied to the seeds as a slurry using an adhesive and the inoculated seeds are covered with finely ground calcium carbonate (Brockwell, 1977).

The production of alginate microbeads is simple and involves a "mist-like" extrusion via a thin nozzle (Bashan and Carrillo, 1996; Carrillo and Bashan, 1997; Stormo and Crawford, 1992). This technology produced alginate beads in sizes of 50 to 200 µm, which entrapped a significant number of bacteria (approx. 10^8 to 10^9 cfu/g), similar to the level obtained in alginate macrobeads (Bashan, 1986a). Application of this novel formulation to the soil is in progress. An alternative to production of microbeads directly is the mechanical crushing of dehydrated macrobeads. to any desired size and their use to inoculate seeds as microbeads (Anonymous, 1995).

So far, it appears that alginate is the most promising of the encapsulating materials tested. However, because of the limited published research on alginate beads in agriculture and because of their possible deficiencies, especially their higher price when compared to peat, it is premature to predict whether alginate will displace peat in the inoculation industry or will remain only in the domain of industrial and environmental microbiology.

Encapsulation With Other Materials

Although commercial alginate preparations are not yet available for bacterial plant inoculation, several other materials, which are used in industrial and environmental microbiology, may be considered as substitutes when the microorganism fails to adapt to alginate preparations. To the best of our knowledge, almost none have been tested in soil or in the field. However, this review should point out their existence to promote further research with these carriers. A list of nine potential

Table 3. Encapsulation and other materials as potential carriers for bacterial inoculants for agriculture other than alginate.

Material	**Advantages**	**Limitations**	**Where used?**	**Reference**
K-carrageenan	It is possible to obtain a higher cell density in the beads than in The original bacterial culture.	The relatively high temperature of bead formation may kill the bacteria .	Yeast for ethanol production, and E. coli, *Seratia marcescens*, and *Acetobacter suboxydans* for L-aspartic acid, L-isoleucine, L-sorbose and ethanol production.	De Taxis Du Poet et al. 1986; Nasri et al. 1987; Wada et al. 1979 1980
Polyacrylamide	Readily available	Very expensive. The monomers are very toxic, and its lowdegradability makes it an environmental liability	Used for encapsulation of *Enterobacter aerogenes* for chorismic acid synthesis or for *Rhizobium* inoculation of legumes	Dommergues et al. 1979; Keller and Lingens, 1984
Agar and agarose	Both are readily available	Very expensive. Relatively high solidification temperature. Slow to degrade.	Encapsulation of *Azospirillum brasilense* and *Pseudomonas fluorescens.*	Bashan, 1982, unpublished data
Xanthan-carob gum	Provides good protection for bacteria	Unknown	Inoculum of *Rhizobium*, as well as *Agrobacterium* and *Arthrobacter.*	Jung et al. 1982; Mugnier and Jun 1985
Polyurethane foam	Not studied	Not studied	Entrapped cells of *Rodococcus chlorophenolicus* and *Flavobacterium sp.* for soil degradation of pentachlorophenol.	Briglia et al. 1990
Vermiculite	Heat sterilized at source; large water-holding capacity, can be prepared in various sizes; cells are held within the porous material and released directly into the soil; does not degrade; eventually incorporates into surrounding soil.	Tends to fall off seeds, does not mix well with seeds, and may accumulate in the planter and stop seed flow.	Used with ectomycorrhizae and *Rhizobium* and commercial formulations have been introduced.	Marx and Kenney, 1982; Paint et al. 1991; Sparrow and Ham 1983 a, b
Polysaccharide adhesive	Not studied	Not studied	Freeze-dried *Bacillus circulans* was added to corn seeds coated with polysaccharide from the same bacteria before sowing.	Berge et al., 1990
Bacterial flocs	Not studied	Not studied	Delivery system for *Azospirillum* and *Rhizobium.*	Neyra et al. 1995

materials for carriers, their basic characteristics, advantages, and limitations is presented in Table 3.

Dried Synthetic Carriers

The main objective of encapsulating bacteria is to increase their survival time during storage (not bacterial number, however, which usually decreases during the process) . Un til now, the most common solutions to this problem of survival time have been air-dried and lyophilized preparations (Bashan 1986a; Bashan et al. 1987; Fages 1992; Kosanke et al. 1992). The lowered water content in the final product is responsible for long-term survival during storage. In this way, the bacteria in the formulation remain inactive, resistant to environmental stresses, insensitive to contamination, and are more compatible with fertilizer application (Paau, 1988).

The dehydration phase is perhaps the *most critical* of the entire formulation process especially for nonspore-forming bacteria. Bacterial survival is affected by several variables: the culture medium used for bacterial cultivation, the physiological state of the bacteria when harvested from the medium, the process of cell encapsulation, the use of protective materials, the type of drying technology used, and the rate of dehydration (Fages 1990; Mary et al. 1985, Paul et al. 1993). If properly dehydrated, the shelf life of the dried formu lation is much longer than that of any peat-based product (Shah-Smith and Burns, 1997).

From both the commercial and the agricultural point of view, the extremely long survival of bacteria in these preparations makes the dry formulations very attractive. By studying the effect of dehydration on encapsulated *Azospirillum* cells, Paul et al. (1993) demonstrated that a large proportion of the cells are destroyed during dehydration. Howevert, when properly dehydrated, the surviving cells are sufficient for inoculation. An additional benefit is that the bacteria survive for almost a year without a drop in population. Dry alginate beads containing *A. brasilense* and *P. fuorescens* produced in 1983 (Bashan 1986a), and which were preserved for archival purposes, maintained their bacterial population for 14 years, although at lower level than at encapsulation time (Bashan, 1997 unpublished).

Alternatively to dried synthetic carrier inoculants, air-dried clay powder with features and advantages similar to these of synthetic carriers (see product section) can be used because, with current technology, it is more cost effective (R.S. Smith, 1995 personal communication).

REGULATION AND CONTROL OF CONTAMINATION OF COMMERCIAL INOCULANTS

Naturally, an inoculant should contain a level of bacteria sufficient to inoculate plants and produce an economic gain. The required level of bacteria cannot be established as a general standard because it varies from one bacterial species to another. Only rhizobial inoculants have

legally established standards. Since this is a new research field, standards for PGPB numbers in inoculants do not yet exist, and every manufacturer can claim whatever he deems appropriate for his product. In fact, some commercial preparations do not even contain any viable cells (Anonymous, 1995; Bashan, 1992 unpublished data).

Quality control methods to determine the number of bacteria within the inoculant are not standardized either. To measure the bacterial number, commonly known methods in microbiology are used; the traditional Plate Count methods, Most Problable Number (Woomer et al. 1990), ELISA, and Immunoblot (Olsen and Rice, 1989; Olsen et al. 1983; Rice and Olsen 1988).

Many developed countries (but not the USA) have regulations for inoculant quality, but in almost all countries in Latin America, inoculant quality is not regulated, nor are the existing regulations well enforced. The level of rhizobia required in the inoculant varies worldwide (between 10^7 and 4×10^9 cfu/g inoculant) and no set of common international standards exists (Olsen et al. 1994a). As far as I know, "Agriculture Canada" is the only governmental agency in North America that has continuously regulated inoculants during the last 20 years. This was after an internal survey by "Agriculture Canada" during the early 1970s which found low levels of *Rhizobium* in commercial preparations (Olsen et al. 1994a). The latest standards in Canada for rhizobia are 10^3 to 10^5 nodulating rhizobia per seed (size-dependent) or 10^{11}, rhizobia per hectare (Bordeleau and Prevost, 1981). Currently, there are only two research stations in Canada which routinely evaluate inoculant quality and publish the results annually. The INRA Laboratory of Soil Microbiology in Dijon plays a similar role in France (J. Fages, 1995 personal communication).

Since the introduction of governmental regulations, there has been an improvement in the quality of commercial inoculants in several countries, including Australia, Canada, and the UK (Brockwell et al. 1988). In most countries, there are no regulations of the level of contaminants in the most commonly used nonsterile peat preparations. A Canadian survey showed the contamination level sometimes exceeded, by several orders of magnitude, the level of rhizobia in the inoculant, indicating poor quality inoculants. In at least one study, it was shown that the contaminant species had detrimental effects on the rhizobia (Olsen et al. 1994b). It is still unknown whether these bacterial contaminants present any health hazards to humans, animals, and plants. These potential hazards should not be ignored as long as nonsterile-carrier inoculants are widely used. These are likely to stay in the market in the future since sterile-carrier inoculants are usually 5 to 10 times higher in price (R.S. Smith, 1995 personal communication). However, one should note that the use of non-sterile carrier inoculants has caused no reported health hazards in decades of usage. France, which has the highest standards for inoculant quality and mandated field testing of new formulations, has a strict requirement that prohibits contaminants in rhizobia inoculants (Catroux, 1991). Australia permits low

levels of contaminants (0.1 % of the total bacterial population), but at the same time requires high population levels of rhizobia (Thompson, 1991). Even some developing countries have very high standards for inoculants. In Rwanda, high rhizobia counts and no more than 0.001% contaminants are allowed (Scaglia, 1991), but it is doubtful whether these high standards are enforced. Surprisingly, the USA and UK have no regulations, perhaps because there have been no reported adverse effects. Inoculant quality control is left to market forces and the manufacturers' discretion (Smith, 1992). The self-imposed standards for rhizobia in American industry are also lower than most European standards. This lack of standard regulation makes it particularly risky for small and medium size growers who tend to purchase inoculants from only one source and may end up with noneffective inoculants (Anonymous, 1995, 1996). Olsen et al (1994a) noted that Canadian regulations sometimes allow even low levels of rhizobia to be legally acceptable, perhaps because the cost of regulation is too high, compared to the risk of misuse. Olsen et al. (1994a) concluded that increased standards not only ensure that the farmer is provided with effective inoculants but are also in the best interest of the inoculation industry. Outlawing low quality inoculants from the market (especially new and lesser known PGPB) will help to prevent a bad public image for the industry and will facilitate the introduction and acceptance of inoculants. It should be noted that the percentage of substandard inoculants in the market is not known, and perhaps the problem is just hypothetical.

Examples of New Commercial Microbial Inoculants

The inoculant industry is not standing still. Its main future prospect is the incorporation of PGPB into products. Apart from *Rhizobium* inoculants, the market for all other bacterial products is smaller than 1% of the respective agrochemical industry. In biologicals, bioinsecticides based on *Bacillus thuringiensis* have a 90% share of the market (and are not within the scope of this review). *Rhizobium* inoculants have the same current dollar value as the bioinsecticides (Lethbridge, 1988).

Numerous manufactures, mainly small to medium size, exist worldwide and have been producing products similar in quality and quantity for decades. For the purpose of illustration only, this review will cite a few examples of these commercial inoculants. A commercial example of a peat-based inoculant is the nonsterile peat granule Soil implant® that still comprises a large portion of the sales of one company (R.S. Smith, 1995 personal communication). Despite the wide use of peat-based inoculants, other types of preparations for rhizobia recently appeared on the market, such as the vermiculite-based Gold Coat™ *Rhizobium* inoculant (Paau et al. 1991), a liquid seed-applied soybean inoculant, Cell-Tech® (Smith, 1995), a liquid in-furrow inoculant LIFT (Smith, 1995) and an air-dried clay powder for alfalfa, Nitragin® Gold (Smith 1995).

Commercial microbial inoculants of other beneficial microorganisms have begun to appear on the market on a small scale. These include "Azogreen", a French-approved *Azospirillum* inoculant

(Fages 1992), Mycostop from Finland, one of the first biofungicides based on *Streptomyces* sp. (Mohammadi, 1994a; Mohammadi and Lahdenperä, 1994), and the biofungicide "Kodiak" based on *Bacillus subtilis* (Backman et al. 1994). Although few strains of PGPB were officially registered in China, many others are being sold off the shelves of research institutes and a few have commercial names [YIB (*Bacillus cerus*); 5406-antagonist (*Streptomyces jingyangensis*)] (Tang and Yang, 1997), identifying them as biocontrol agents and as growth promoters. In Japan, a product based on *Agrobacterium radiobacter* K84 (Bakuterohzu) to control crown gall in rose was registered (Tsuchiya, 1997). Its Australian predecessor, NoGall, has been on the market for years (Kerr, 1989). BioCoatTm, a product of *P. fuorescens* for suppression of *Fusarium* sp. in radish, was phased out in 1996 after several years of use, because of low disease suppression and short shelf-life (Kempf et al, 1997). Several other PGPB products are currently in the pipe-line for final release (Mariano et al, 1997; Kikumoto, lecture at the 4th International Workshop on Plant Growth-Promoting Rhizobacteria, Sapporo, Japan, 1997).

TIME AND APPLICATION METHODS FOR BACTERIAL INOCULANTS

A major advantage of formulated bacteria is that they are usually readily applied using standard farming machinery. It is unlikely, even in developed countries, let alone in developing countries, that farmers will purchase specialized equipment to test an unproven product. Additionally, ease of use and the absence of time-consuming application procedures are also major factors.

There are several common methods of inoculant application, yet farmers are not always willing to practice them. The primary limiting factors in developed countries are the cost of labor and time pressures at planting (Smith 1992). In developing countries, there is insufficient knowledge, lack of adequate machinery, and improper distribution and importation laws for live inoculants that can lose their viability and effectiveness (Anonymous, 1995). In North America, for example, Smith (1992) wrote that farmers follow recommended procedures *only* if they have plenty of time for planting. This is particularly difficult for large growers in temperate zones who need to sow in a limited period of time. Relatively laborious methods such as the recommended slurry method are phased out for less laborious ones like the common dry method. Farmers are discouraged from inoculant use if they have to make additional passes over the sown field.

Two main methods of inoculation are currently being used: secd inoculation and soil inoculation (Hegde, 1992). The latter is done by delivering the inoculant directly into the sowing furrow with the seeds (Gault, 1982). Seed inoculation is the most popular method worldwide, as long as the farmer is willing to take the extra step of mixing the inoculant with the seeds immediately

Table 4. Methods of inoculation with peat-based inoculants

Method	Advantages	Disadvantages	Reference
1. Seed inoculation with powder formulations	When properly applied, it ensures that each seed receives the introduced bacteria	The quantity of bacteria that can be attached to the seed surface is limited, especially for small seeds	Brockwell et al. 1977; Smith, 1992
	A small quantity of inoculant is used	Bacteria may have direct contact with pesticides applied to seeds	Kosanke et al. 1992
	Seed precoated with inoculant provide a convenience to the farmer who purchases seeds that have already been inoculated	Possibility of movement of the inoculant upward with the cotyledons instead of downward with the roots	
	The inoculant can be applied by the farmer	The inoculant provides no protection against stress conditions (drought, high temperatures)	
		Inoculant provides little or no protection for the bacteria from desiccation before planting. Requires the addition of a sticky substance; an extra step in the field that is usually ignored by e wets.	
1.1 "Dry Mixing" (dry inoculant mixed with the seeds in the seed hopper)	The inoculant is very convenient for the grower and therefore, popular	Seed adhesion is poor and much of the inoculant is lost during mixing and application	
1.2 "Sprinkle method" (a small amount of water mixed with seeds before peat powder is added and mixed)		Upon desiccation, a considerable amount of inoculant falls from the seeds	
1.3 "Slurry method" (The inoculant is suspended in water, added to the seeds and mixed).	Upon drying, the inoculant remains on the seed especially if no adhesive is added		
1.4 "Seed pelleting"	Inoculant contains high populations of bacteria. Provides very good survival of the bacteria in the inoculant.	Procedure must be done by seed-processing company, which increases the price	

(Table continued)

(Table continued)

Inoculant + adhesive are applied as slurry to seeds and coated with ground material like lime.	Procedure can neutralize the effect of acid soil around the seed	Difficult, time consuming and relatively expensive industrial process	
2. Peat suspension in water sprayed into the furrow during sowing.	A convenient procedure that is an alternative to seed-applied peat powder		
	Increases inoculation rates by increasing either the amount of water or bacteria		Brockwell et al. 1988; Gault et al. 1982
3. Liquid formulations (application to the seeds and mixing before planting)	Convenient to the grower, very good seed coverage and seed adhesion	Does not shield the bacteria from the soil environment and microfloral predation or assist bacterial survival under stress conditions	
4. Soil inoculation with peat ales.	Greater delivery quantities can be used (than in seed coating)	Application has a higher cost than seed coating	
(The most common method of "in-furrow" application)	Provides minimal contact with chemicals applied to the seeds	After releasing from the granules, the inoculum is subjected to competition with native soil microbes	Bashan et al. 1987
	Eliminates the field work of mixing seeds with inoculant	Once out of the granules, the inoculum can be preyed on by protozoa	Bezdicek et al. 1978
	Increases the bacterial ability to withstand low moisture conditions	To colonize roots, the inoculant must move through the soil from the inoculation site to the roots	Rice and Olsen, 1988
	Easy to use with agricultural granular applicators		
	Better nodulation and yields in legumes than inoculation with peat powder		

before sowing. The less common method, soil inoculation, is now being used successfully for rhizobia inoculation, but has several disadvantages which limit its future for the application of *Azospirillum*, which survives poorly in many soils (Bashan et al. 1995).

By analyzing the advantages and disadvantages of the two methods (Table 4), it can be concluded that while there is currently room for both methods, the future probably lies with seed inoculation. For inoculation of soybeans, currently the major inoculated crop, changing management practices (conservation tillage and narrow rows) also limit the use of the granular form (R.S. Smith, 1995 personal communication).

Microbial inoculants can be applied during three possible phases: (i) at the seed processing plant as a seed coating, months before the actual sowing, (ii) "on site", as a seed application just before sowing, or by inoculant delivery directly onto the seeds in the furrow, and (iii) after seedlings emerge (Bashan, 1986b). The most popular method to date with peatbased inoculants is the "on site" method, primarily because of lower costs. Fages (1992) pointed out some major drawbacks for "on-site" seed inoculation: (i) additional work is required during sowing, which is time restricted, (ii) the seed germination rate may decrease if some seeds are damaged during the mixing step with the inoculant, (iii) since the bacteria in the inoculant are alive, they may be subjected to UV irradiation which can reduce their population during the field mixing operation, and (iv) the bacterial population may be reduced when the wet inoculant is attached to the chemically coated seeds. All these drawbacks also exist when seeds are precoated by industry, albeit to a lesser extent.

Although it requires a more expensive technology, the precoating of seeds merits more research to eliminate all or most of the above mentioned difficulties (Paul et al. 1991), primarily because it is most convenient for the farmer. Of particular interest is the interaction between the seed and the bacterial cells. Polysaccharide (either natural or synthetic) matrixes, which can be attached harmlessly and easily to the seeds, are the most promising materials to be evaluated (Berge et al. 1990).

Soil inoculation is an alternative to seed inoculation. It is more convenient for the farmer than seed inoculation, but is sometimes not as effective. It is also more expensive because more inoculant is required. Soil inoculation can be done either with peat-based granules or with micrograniulated forms of inert materials; sand, calcium carbonate, or marble powder. These materials have been previously mixed with the inoculum in the factory or can be mixed with the seeds by the farmer prior to sowing. The technique uses a specific granular applicator which makes use of insecticide applicators farmers already. have. Fages (1994) reported that a commercial product for *A. lipoferum* has successfully used this technology with corn for four years in 13 locations in France. Alternatively, liquids applied in furrow are also available for legume inoculation (Anonymous, 1996).

It is clear that some applications use a large amount of inoculant (by weight or volume), up to several tons of inoculant per hectare. This makes them uneconomical for many crops. It is expected that such bulky inoculants will be phased out of the market soon. In the agrochemical industry, application levels (of active ingredient and water) are low and decreasing. The new bacterial inoculants must meet these standards if they are to compete with chemicals on the farmer's list.

COST OF DEVELOPMENT AND MARKETING

The cost of developing a new product by the agrochemical industry has been estimated at over $80 million US and rising (Wood-Mackenzie, 1987, cited in Lethbridge, 1988). The development of resistance to pesticides may shorten the commercial life of these products and thus their potential return. The development of bacterial inoculants is claimed to be cheaper than that of agrochemicals, although the large scale screening of strains with biological activity is still required (comparable to more than 1:20,000 screened molecules for a new chemical product) (Lam, S. 1997. Lecture in the 4th International Workshop on Plant Growth-promoting Rhizobacteria, Sapporo, Japan). However, having a predetermined pathogen as a research goal will dictate where to look for its antagonist, making the initial screening easier. In the case of a biocontrol PGPB, it has been shown that it is rather easy to detect a potential antagonist in vitro (Kloepper 1994; Kloepper et al. 1989; Glick, 1995). The following are some factors that reduce the costs of development of bacterial inoculants which makes them attractive to the agrochemical industry: (i) reduced registration costs compared to those of chemical-product test programs that are well- established and costly. The cost of registration of naturally-occurring bacteria is relatively low (Markle, 1983) because the registration agencies worldwide expect a lower environmental impact from an indigenous microorganism, even in large quantities, than from a man-made, and possibly toxic, molecule. This does not consider the cost of registration of genetically engineered bacteria since those regulations are still being formulated by agencies in each country (Del Bino, 1991; Shaw et al. 1992); (ii) reduced registration time decreases the time span from first screening to market, thus increasing revenues; (iii) the possibility of developing bacterial products for small markets. Since the cost involved in bringing a new chemical to the marketplace is so large, the product must be targeted to a market large enough to have a good return on investment. This limits the choice of crops to the major crops only. With a cheaper bacterial inoculant, the smaller markets of site-specific agriculture become available (Robert and Rust, 1995). Yet, one should bear in mind that this type of future agriculture will probably increase the cost of development of bacterial inoculants, since many types of inoculants should be developed; and (iv) although fermentation is costlier than chemical production, the fermentation plant is more versatile.

It can produce, with minor modifications, different products rather then specializing in a single chemical formulation.

Other incentives for the agrochemical industry to develop bacterial inoculants might be: (i) It is less likely that pathogens will develop resistance as fast as they do to chemical products. However, some bacterial inoculants, especially those that use an organism employing a single mechanism against the pathogen, can also develop resistance. (ii) They are "environmentfriendly". The "natural" tag of bacterial inoculants (especially those that are nonengineered and indigenous) make them more acceptable in the public eye, and especially to the "Green movement" pressure groups, than chemicals. Although theoretically true, this is not always necessarily so, i.e., the acceptance of *B. thuringiensis* as safe for humans and the environment occurred only after the product's effectiveness was confirmed at a cost equivalent to the chemical alternative (Kenney, 1997). On the other hand, a "natural" label by itself will not guarantee acceptance of a mildly effective product in the market place (Lethbridge, 1988).

Regarding the commercialization of inoculant products, any inoculant R&D laboratory should consider the guidelines of Lethbridge (1988), Fages (1992) and Kenney (1997). First, all the considerations mentioned above (efficient strains, optimized formulations, cost-effective production, and good and practical inoculation techniques) are not sufficient to launch a new product on the market nor guarantee its success. The following practical variables should be considered: (i) the product must be efficient and reliable in large-scale field trials and especially under "real life" conditions; the value to the grower is the number one factor of success of any new inoculant, (ii) registration costs and delays that vary from one country to another (Mohamaddi 1994b), (iii) obviously, patents on industrial processes and registration of biological products must be secured (patent registration is time consuming and expensive in most countries albeit less tedious than the registration of chemicals), (iv) for every potential customer country, a market survey must be done which examines customer demand, market size, and expected selling price, (v) safety concern and environmental consciousness are markedly more important today than they were in the past, (vi) the ease of handling of an inoculant is .a major issue for the end users; the need to keep an inoculant refrigerated has an adverse effect on its commercialization, and (vii) the biological product must have the ability to be compatible with chemicals used in Integrated Pest Management which today is the common state of the art of pest control.

CONCLUSIONS AND FUTURE PROSPECTS

Microbial inoculants have long been incorporated into field practices worldwide, with satisfactory results, especially for rhizobia (Scott, 1965; Smith et al. 1981; Somasegaran, 1985). Compared with chemical applications in agriculture, their present impact on the agromarket is

small. However, the agrochemical industry is more sympathetic now to the concept of bacterial inoculants than it has been previously. There is a genuine interest in developing bacterial products that are reliable and that can act as complements to chemicals already on the market (Anonymous, 1995). Research and limited field trials of PGPB over the last decade have opened up new horizons for the inoculation industry (Bashan et al. 1989, Bashan and Levanony, 1990; Kloepper et al. 1989). It is relatively easy to isolate a bacterial antagonist to a phytopathogen (Bashan et al. 1993), or to find a bacterium that will increase root development (Glick et al. 1995). Yet, methods to identify the best bacteria for the task are little known (Glick, 1995), and even less is known about their rhizocompetence and other characteristics required of potentially beneficial bacteria to survive, and function in their new environment (Bashan and Holguin, 1997a.b; Hagedorn, 1993). An additional challenge is to develop improved carriers that consistently provide higher bacterial numbers under field conditions, extended shelf life, protection against the soil environment, convenience of use, and cost effectiveness (Smith 1992).

Most inoculants today are used for legumes and to a lesser extent for cereals (Anonymous, 1995). The market dictates that the inoculant must be as cheap as possible. The cost of developing new inoculant materials quickly moves the price out of a practical range for agriculture, especially in developing countries. However, there are several high-value specialty markets such as flowers, fresh organic fruits and vegetables, where chemicals are undesirable or become difficult to use because of restrictions. Greenhouse crops are also primary targets for commercial inoculants. Since they are often grown in disinfected soils or even without soil but with high input costs, the additional inoculation costs will not cause an unacceptable economic burden to the grower. At the same time, this type of cultivation avoids all the difficulties originating from the interaction of the inoculants with the soil (Fages, 1995 personal communication). Therefore, these markets, if developed properly, can represent an opportunity for novel PGPB inoculants.

It appears that for legume inoculation with rhizobia, developing inoculants will be the best option for some time to come, since nitrogenase expression in plants is unlikely to occur in the foreseeable future (Glick and Pasternak, 1994). However, for PGPB operating via other mechanisms, there is a theoretical option that may render the entire inoculation industry, apart from that for rhizobia, obsolete; namely the possibility of engineering plants containing bacterial mechanisms. Such pioneering transgenic plants are already in the field expressing insecticidal proteins of *B. thuringiensis* in cotton plants, making them resistant to various insect pests (Benedict et al. 1996; Perlak et al. 1990). If this novel approach materializes on a large scale with PGPB (Tsuchiya, 1997), most of the concerns expressed in this review about inoculants, formulations, and grower acceptance will vanish. However, one concern still remains even with this approach: PGPB may function through multiple mechanisms. Transfer of a single mechanism may not provide significant benefits. With engineered

crops, most of the technical difficulties inherent in bacterial inoculants are removed because the grower simply purchases the "modified" seeds, which certainly will be more expensive. Therefore, although identification of desirable bacterial mechanisms and their mobilization into the plant genome is outside of the scope of this review, this approach to agricultural improvement should be considered, especially with the heavy emphasis it is currently receiving in industrial research (Anonymous, 1996).

During the last century, peat formulations have been developed into effective and accepted carriers, but their development has almost reached its limits. Synthetic carriers, which have yet to be transferred from experimental concepts into commercial inoculants, offer greater potential and flexibility for the inoculation industry. Due to the shortage of information about new developments from inoculant companies, it is premature to view these carriers as potentially universal, even though they overcome many of deficiencies of peat-based inoculants.

While it is true that in contemporary agricultural practices synthetic inoculants are frequently too expensive for the target crop, and therefore companies are reluctant to develop them, the bioremedation industry might support development of such advanced inoculants. Many types of encapsulated forms of microorganisms have been developed for bioremedation use (Cassidy et al. 1996). Many bioremedation projects are supported by governments in developing countries or by large contaminating industries in developed countries forced to "clean up"; both of which are more resourceful than an individual farmer. More efficient inoculants will undoubtedly be used for bioremedation processes, especially in emergencies, regardless of their higher costs. This use may provide agriculture with the development of novel inoculant materials and formulations. A wider use in nonagricultural applications may help these materials become cost competitive for agriculture (Anonymous 1996).

From a realistic perspective, one must accept that, in the foreseeable future, chemicals will continue to dominate the market. Only a gradual and modest increase in the use of bacterial inoculants is to be expected. Agriculture in developed countries is definitely the major promoter of microbial inoculants that are "environmentally friendly". Nevertheless, special attention should be paid to the needs and constraints of developing countries that need easy-to-use and inexpensive formulations.

For the short-and medium-term future, more research should focus on the development of better and more economical feasible, synthetic inoculant carriers, while sustaining peat-based inoculant production for agriculture. The other options should be considered as long-term goals.

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