Chapter 10

PLANT GROWTH PROMOTING RHIZOBACTERIA FORMULATIONS AND ITS SCOPE IN COMMERCIALIZATION FOR THE MANAGEMENT OF PESTS AND DISEASES

S. NAKKEERAN[1], W. G. DILANTHA FERNANDO[1] AND ZAKI A. SIDDIQUI[2]

[1]Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2; [2]Department of Botany, Aligarh Muslim University, Aligarh-202002,India

Abstract: The export oriented agricultural and horticultural crops depends on the export of residue free produce and has created a great potential and demand for the incorporation of biopesticides in crop protection. To ensure the sustained availability of biocontrol agent's mass production technique and formulation development protocols has to be standardized to increase the shelf life of the formulation. It facilitates the industries to involve in commercial production of plant growth promoting rhizobacteria (PGPR). PGPR with wide scope for commercialization includes *Pseudomonas fluorescens, P. putida, P. aeruginosa, Bacillus subtilis* and other *Bacillus* spp. The potential PGPR isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. They are delivered either through seed treatment, bio-priming, seedling dip, soil application, foliar spray, fruit spray, hive insert, sucker treatment and sett treatment. Application of PGPR formulations with strain mixtures perform better than individual strains for the management of pest and diseases of crop plants, in addition to plant growth promotion. Supplementation of chitin in the formulation increases the efficacy of antagonists. More than 33 products of PGPR have been registered for commercial use in greenhouse and field in North America. Though PGPR has a potential scope in commercialization, the threat of certain PGPR (*P. aeruginosa*, *P. cepacia* and *B. cereus*) to infect human beings as opportunistic pathogens has to be clarified before large scale acceptance, registration and adoption of PGPR for pest and disease management.

Key words: biocontrol; biopesticides; commercialization; formulations; PGPR.

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1 INTRODUCTION

Despite the use of available means of plant protection, about one third of the crops produced are destroyed by pests and diseases. The discovery of synthetic chemicals has contributed, greatly to the increase of food production industry by controlling pests and diseases. However, the use of these synthetic chemicals during the last three decades has raised a number of ecological problems. In the recent years, scientists have diverted their attention towards exploring the potential of beneficial microbes, for plant protection measures. Bio-control agents are easy to deliver, improve plant growth, and activate resistance mechanism in the host, and increase biomass production and yield. These antagonists act through antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism and through competition for space and nutrients.

Though bio-control with PGPR is an acceptable green approach, the proportion of registration of biocontrol agents for commercial availability is very slow. In addition, the present day bio-products can be further improved to obtain greater levels of disease reduction. Development of formulations with increased shelf life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate.

2 CHARACTERISTICS OF A SUCCESSFUL PGPR FOR FORMULATION DEVELOPMENT

To develop a successful PGPR formulation, rhizobacteria should possess

a. High rhizosphere competence
b. High competitive saprophytic ability
c. Enhanced plant growth
d. Ease for mass multiplication
e. Broad spectrum of action
f. Excellent and reliable control
g. Safe to environment
h. Compatible with other rhizobacteria
i. Should tolerate desiccation, heat, oxidizing agents and UV radiations (Jeyarajan and Nakkeeran, 2000).

3 FORMULATION DEVELOPMENT

Major research on biocontrol is centered with the use of cell suspensions of PGPR directly to seed. Technologies become viable only

when the research findings are transferred from the lab to field. Though PGPR have a very good potential in the management of pests and diseases, it could not be used as cell suspension under field conditions. Hence, the cell suspensions of PGPR should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization and field use.

4 CHARACTERISTICS OF AN IDEAL FORMULATION

a. Should have increased shelf life
b. Should not be phytotoxic to the crop plants
c. Should dissolve well in water and should release the bacteria
d. Should tolerate adverse environmental conditions
e. Should be cost effective and should give reliable control of plant diseases
f. Should be compatible with other agrochemicals
g. Carriers must be cheap and readily available for formulation development (Jeyarajan and Nakkeeran, 2000).

5 CARRIERS IN FORMULATION DEVELOPMENT

Commercial application of PGPR either to increase crop health or to manage plant diseases depend on the development of commercial formulations with suitable carriers that support the survival of bacteria for a considerable length of time. Carriers may be either organic or non-organic. It should be economical and easily available.

5.1 Organic/Non-organic Carriers

The organic carriers used for formulation development include peat, turf, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, pressmud, sawdust, and vermiculite, etc. Carriers increase the survival rate of bacteria by protecting it from desiccation and death of cells (Heijnen *et al*., 1993). The shelf life of bacteria varies depending upon bacterial genera, carriers and their particle size (Table 1). Survival of *P. fluorescens* (2-79RN10, W4F393) in montmorillonite, zeolite and vermiculite with smaller particle size increased the survival rate than in kaolinite, pyrophyllite and talc with bigger particle size. The carriers with smaller particle size have increased surface area, which increase resistance

to desiccation of bacteria by the increased coverage of bacterial cells (Dandurand *et al*., 1994).

5.1.1 Talc / Peat / Kaolinite / Lignite / Vermiculite based formulations

Formulations of fluorescent Pseudomonas were developed through liquid fermentation technology. The fermenter biomass was mixed with different carrier materials (Talc/ Peat/ Kaolinite/ Lignite/ Vermiculite) and stickers (Vidhyasekaran and Muthamilan, 1995). Krishnamurthy and Gnanamanickam (1998) developed talc based formulation of *P. fluorescens* for the management of rice blast caused by *Pyricularia grisea*, in which methyl cellulose and talc was mixed at 1: 4 ratio and blended with equal volume of bacterial suspension at a concentration of 10^{10}cfu/ml. Nandakumar *et al*. (2001) developed talc based strain mixture formulation of fluorescent pseudomonads. It was prepared by mixing equal volume of individual strains and blended with talc as per Vidhyasekaran and Muthamilan (1995). Talc based strain mixtures were effective against rice sheath blight and increased plant yield under field conditions than the application of individual strains. Talc and peat based formulations of *P. chlororaphis* and *B. subtilis* were prepared and used for the management of turmeric rhizome rot (Nakkeeran *et al*., 2004).

One school of thought explains that CMC is added as a sticker at 1: 4 ratio to talc. Though it is effective in disease management, it would lead to the increase in the production cost, which would prevent the growers to adopt the technology. More over another school of thought explain that CMC and talc should be used at 1:100 ratios. Hence feasibility of the technique and shelf life of the product has to be evaluated to make the technology as a viable component in disease management so as to promote organic farming.

5.1.2 Microencapsulation

Microcapsules of rhizobacteria consists of a cross linked polymer deposited around a liquid phase, where bacteria are dispersed. Microparticles are characterized based on the distribution of particle size, morphology and bacterial load. The process of microencapsulation involves mixing of gelatin polyphosphate polymer pair (81:19 w/w) at acidic pH with rhizobacteria suspended in oil (Charpentier *et al*., 1999). Though rhizobacteria has been formulated through microencapsulation method, its shelf life declines at a faster rate, since polymers serve as a barrier for oxygen. This was later improved by developing microcapsules by spray drying. The release of *P. fluorescens-putida* from the microencapsulated pellets occurred after 15

min immersion in aqueous buffer. It showed that water served as triggering material for the bacterial release (Charpentier *et al*., 1999).

Though, microencapsulation aids in formulating bacteria, still the technology has to be well refined for early release of bacterial cells and for the establishment in the infection court to counter attack the establishment of pathogens. Most of the experiments on microencapsulation have been restricted only to lab. The technology should be standardized for the industrial application so that the technical feasibility could be assessed to popularize the same for field use.

6 FORMULATIONS AND SHELF LIFE

6.1 Talc formulation

Talc is a natural mineral referred as steatite or soapstone composed of various minerals in combination with chloride and carbonate. Chemically it is referred as magnesium silicate (Mg3Si4O10(OH)2 and available as powder form from industries suited for wide range of applications. It has very low moisture equilibrium, relative hydrophobicity, chemical inertness, reduced moisture absorption and prevent the formation of hydrate bridges that enable longer storage periods (http://www.luzenac.com/food.htm). Owing to the inert nature of talc and easy availability as raw material from soapstone industries it is used as a carrier for formulation development.

Kloepper and Schroth (1981) demonstrated the potentiality of talc to be used as a carrier for formulating rhizobacteria. The fluorescent Pseudomonads did not decline in talc mixture with 20% xanthum gum after storage for two months at 4°C. *P. fluorescens* isolate Pf1 survived up to 240 days in storage. The initial population of Pf1 in talc-based formulation was 37.5 x 10^7cfu/g and declined to 1.3 x 10^7cfu/g after 8 months of storage (Vidhyasekaran and Muthamilan, 1995). Amendment of sucrose (0.72M) in King's B medium increased population and shelf life of *P. fluorescens* (P7NF, TL3) in talc-based formulation up to 12 months (Caesar and Burr, 1991). *P. putida* strain 30 and 180 survived up to 6 months in talc based formulations. The population load at the end of 6^{th} month was 10^8 cfu/g of the product (Bora *et al*., 2004).

6.2 Peat formulations

Peat (Turf) is a carbonized vegetable tissue formed in wet conditions by decomposition of various plants and mosses. It is formed by the slow decay of successive layers of aquatic and semi aquatic plants, e.g., sedges,

reeds, rushes, and mosses. Peat soils are used as carrier materials to formulate PGPR. Though peat carriers are cheap to use, it harbors lot of contaminants. The quality of peat is variable and not readily available worldwide. Sterilization of peat through heat releases toxic substances to the

Table 1. Shelf life of formulations in different carrier materials

Carrier	**Bacteria**	**Shelf life**	**Reference**
Talc	Rhizobacteria	2 months	Kloepper and Schroth (1981)
Talc	*P. fluorescens* (P7NF, TL3)	12 months (8.4 Log cfu/g)	Caesar and Burr (1991)
Talc	*P. fluorescens* (Pf1)	8 months (1.3 x 10^7cfu/g)	Vidhyasekaran and Muthamilan (1995)
Talc	*B. subtilis*	45 days (1.0 x 10^6 cfu/g)	Amer and Utkhede (2000)
Talc	*P. putida*	45 days (1.0 x 10^3 cfu/g)	Amer and Utkhede (2000)
Talc	*P. putida* strain 30 and 180	6 moths (>1 x 10^8cfu/g) (not estimated during subsequent months)	Bora *et al*. (2004)
Lignite	*P. fluorescens* (Pf1)	4 months (2.8 x 10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Peat	*P. fluorescens* (Pf1)	8 months (7.0 x 10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Peat supplemente-d with chitin	*B. subtilis*	6 moths (>1 x 10^9cfu/g) (not estimated during subsequent months)	Manjula and Podile (2001)
Peat	*P. chlororaphis* (PA23) and *B. subtilis* (CBE4)	6 moths (>1 x 10^8cfu/g) (not estimated during subsequent months)	Nakkeeran *et al*. (2004)
Vermiculite	*P. fluorescens* (Pf1)	8 months (1.0 x 10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Vermiculite	*B. subtilis*	45 days (>1.0 x 10^6 cfu/g)	Amer and Utkhede (2000)
Vermiculite	*P. putida*	45 days (>1.0 x 10^3 cfu/g)	Amer and Utkhede (2000)
Farm yard manure	*P. fluorescens* (Pf1)	8 months (1.0 x 10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)
Kaolinite	*P. fluorescens* (Pf1)	4 months (2.8 x 10^6cfu/g)	Vidhyasekaran and Muthamilan (1995)

bacteria and there by reduce bacterial viability (Bashan, 1998). Peat based formulation of *Azospirillum brasilense* had a shelf life up to 4 months. The population load after 4 months of storage was 10^7 cfu/g of the product (Bashan, 1998). This population was sufficient for successful plant inoculation (Garcia and Sarmiento, 2000). Vidhyasekaran and Muthamilan

(1995) reported that the shelf life of *P. fluorescens* in peat-based formulation was maintained up to 8 months (2.8 x 10^6cfu/g). Shelf life of *P. chlororaphis* (PA23) and *B. subtilis* (CBE4) in peat carriers was retained for more than six months (Kavitha *et al*., 2003; Nakkeeran *et al*., 2004).

6.3 Press mud formulation

Press mud is a byproduct of sugar industries. It was composted using vermin-composting technique and later used as a carrier for *Azospirillum* spp. This carrier maximizes the survival of *Azospirillum* spp than lignite, which is predominantly used as a carrier material in India (Muthukumarasamy *et al*., 1997).

6.4 Vermiculite formulation

Vermiculite is a light mica-like mineral used to improve aeration and moisture retention. It is widely used as potting mixture and used as a carrier for the development of formulations for harboring microbial agents. Vermiculite based formulation of *P. fluorescens* (PF1) retained shelf life for a period of 8 months. The viable load of bacteria in the formulation was 1x10^6cfu/g (Vidhyasekaran and Muthamilan, 1995). Shelf life of *Azospirillum* in vermiculite-based formulation was retained up to 10 months. The viable cells after 44 weeks of storage were 1.3 x 10^7 cfu/g (Saleh *et al*., 2001).

7 DELIVERY SYSTEMS

Plant growth promoting rhizobacteria are delivered through several means based on survival nature and mode of infection of the pathogen. It is delivered through seed, soil, foliage, rhizomes, setts, or through combination of several methods of delivery.

7.1 Seed treatment

Seed treatment with cell suspensions of PGPR was effective against several diseases. Delivering of *Serratia marcescens* strain 90-166 as seed dip before planting and soil application of 100 ml of the same at the rate of 10^8 cfu/ml to the sterilized soil less planting mix after seeding reduced bacterial wilt of cucumber and controlled cucumber beetles besides increasing the fruit weight (Zehnder *et al*., 2001). Transfer of technology for commercial use could be possible if PGPR strains are made available as a product. After

realization of the same, several carriers were used for formulation development. Talc based formulation of *P. fluorescens* Pf1 was coated on to seeds at the rate of 4g/Kg (10^7cfu/g) of chickpea seeds (cv.Shoba) for the management of chickpea wilt. Sowing of treated chickpea seeds resulted in establishment of rhizobacteria on chickpea rhizosphere (Vidhyasekaran and Muthamilan, 1995). Treatment of cucumber seeds with strain mixtures comprising of *Bacillus pumilus* - INR7, *B. subtilis* – GB03 and *Curtobacterium flaccumfaciens* – ME1 with a mean bacterial density of $5x10^9$cfu/seed reduced intensity of angular leaf spot and anthracnose equivalent to the synthetic elicitor Actigard and better than seed treatment with individual strains (Raupach and Kloepper, 1998). Treatment of pigeonpea seeds with talc based formulation of *P. fluorescens* (Pf1) effectively controlled fusarial wilt of pigeonpea under greenhouse and field conditions (Vidhyasekaran *et al*., 1997). Soaking of rice seeds in water containing 10g of talc based formulation of *P. fluorescens* consisting mixture of PF1 and PF2 (10^8cfu/g) for 24h controlled rice sheath blight under field condition (Nandakumar *et al*., 2001). Seed treatment of lettuce with either vermiculite or kaolin based carrier of *B. subtilis* (BACT-0) significantly reduced root rot caused by *P. aphanideramtum* and it also increased the fresh weight of lettuce under greenhouse conditions. Seed treatment with vermiculite based *P. putida* reduced fusarium root rot of cucumber and increased the yield and growth of cucumber (Amer and Utkhede, 2000). Treatment of tomato seeds with powder formulation of PGPR (*B. subtilis, B. pumilus*) reduced symptom severity of ToMoV and increased the fruit yield (Murphy *et al*., 2000).

7.2 Bio-priming

A successful antagonist should colonize rhizosphere during seed germination (Weller, 1983). Priming with PGPR increase germination and improve seedling establishment. It initiates the physiological process of germination, but prevents the emergence of plumule and radicle. Initiation of physiological process helps in the establishment and proliferation of PGPR on the spermosphere (Taylor and Harman, 1990). Bio-priming of seeds with bacterial antagonists increase the population load of antagonist to a tune of 10 fold on the seeds thus protected rhizosphere from the ingress of plant pathogens (Callan *et al*., 1990). Chickpea seeds treated with talc-based formulation of Pf1 was primed by incubating the treated seeds for 20h at 25°C over sterile vermiculite moistened with sterile water. Population of Pf1 increased up to 100% in the rhizosphere, indicating that it provides a congenial microclimate for proliferation and establishment of bacterial antagonist (Vidhyasekaran and Muthamilan, 1995). Drum priming is a

commercial seed treatment method followed to treat seeds with pesticides. Drum priming of carrot and parsnip seeds with *P. fluorescens* Pf CHAO proliferated well on the seeds and could be explored for realistic scale up of PGPR (Wright *et al*., 2003).

7.3 Seedling dip

PGPR is delivered through various means for the management of crop diseases based on the survival nature of pathogen. In several crops pathogens gain entry into plants either through seed, root or foliage. In rice, sheath blight incited by *Rhizoctonia solani* is a major obstacle in rice production. As the pathogen is soilborne, it establishes host parasite relationships by entering through root. Hence, protection of rhizosphere region by prior colonization with PGPR will prevent the establishment of host-parasite relationship. Delivering of *P. fluorescens* strain mixtures by dipping the rice seedlings in bundles in water containing talc based formulation of strain mixtures (20g/l) for 2h and later transplanting it to the main field suppressed sheath blight incidence (Nandakumar *et al*., 2001). Similarly dipping of rice seedlings in talc based formulation of *P. fluorescens* (PfALR1) prior to transplanting reduced sheath blight severity and increased yield in Tamil Nadu, India (Rabindran and Vidhyasekaran, 1996). Dipping of strawberry roots for 15 minutes in bacterial suspension of *P. putida* (2 x 10^9 cfu/ml) isolated from strawberry rhizosphere reduced *Verticillium* wilt of strawberry by 11% compared to untreated control (Berg *et al*., 2001). Dipping of *Phyllanthus amarus* seedlings in talc based formulation of *B. subtilis* (BSCBE4) or *P. chlororaphis* (PA23) for 30 minutes prior to transplanting reduced stem blight of *P. amarus* (Mathiyazhagan *et al*., 2004).

7.4 Soil application

Soil being as the repertoire of both beneficial and pathogenic microbes, delivering of PGPR strains to soil will increase the population dynamics of augmented bacterial antagonists and thereby would suppress the establishment of pathogenic microbes on to the infection court. Vidhyasekaran and Muthamilan (1995) stated that soil application of peat based formulation of *P. fluorescens* (Pf1) at the rate of 2.5 Kg of formulation mixed with 25 Kg of well decomposed farm yard manure; in combination with seed treatment increased rhizosphere colonization of Pf1 and suppressed chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris*. Broadcasting of talc based formulation of strain mixtures (Pf1 and FP7) by blending 2.5 kg of formulation with 50 kg of sand after 30 days of

transplanting paddy seedlings to main field significantly reduced sheath blight and increased yield under field conditions (Nandakumar *et al*., 2001). Incorporation of commercial chitosan based formulations LS254 (comprising of *Paenobacillus macerans* + *B. pumilus*) and LS255 (comprising of *P. macerans* + *B. subtilis*) into soil at the ratio of 1: 40 (Formulation: Soil) increased bio-matter production by increasing both root and shoot length and yield (Vasudevan *et al*., 2002). Soil application of the strain mixture formulations LS256 and LS257 comprising of two different *Bacillus* spp., was better than seed treatment and suppressed downy mildew under greenhouse and field conditions (Niranjan Raj *et al*., 2003).

7.5 Foliar spray

The efficacy of biocontrol agents for foliar diseases is greatly affected by fluctuation of microclimate. Phyllosphere is subjected to diurnal and nocturnal, cyclic and non-cyclic variation in temperature, relative humidity, dew, rain, wind and radiation. Hence water potential of phylloplane microbes will be varying constantly. It will also vary between leaves or the periphery of the canopy and on sheltered leaves. Higher relative humidity could be observed in the shaded, dense region of the plant than that of peripheral leaves. The dew formation is greater in centre and periphery. The concentration of nutrients like amino acid, organic acids and sugars exuded through stomata, lenticels, hydathodes and wounds varies highly. It affects the efficacy and survival of antatognist in phylloplane (Andrews, 1992).

Delivering of *Pseudomonas* to beet leaves actively compete for amino acids on the leaf surface and inhibited spore germination of *Botrytis cinerea*, *Cladosporium herbarum* and *Phoma betae* (Blakeman and Brodie, 1977). Application of *B. subtilis* to bean leaves decreased incidence of bean rust (*Uromyces phaseoli)* by 75% equivalent to weekly treatments with the fungicide mancozeb (Baker *et al*., 1983). Application of *P. fluorescens* on to foliage (1kg of talc based formulation /ha) on 30, 45, 60, 75 and 90 days after sowing reduced leaf spot and rust of groundnut under field conditions (Meena *et al*., 2002). Preharvest foliar application of talc based fluorescent pseudomonads strain FP7 supplemented with chitin at fortnightly intervals (5g/l; spray volume 20l/ tree) on to mango trees from pre-flowering to fruit maturity stage induced flowering to the maximum, reduced the latent infection by *C. gloeosporioides* beside increasing the fruit yield and quality (Vivekananthan *et al*., 2004). Though seed treatment and foliar application of *P. fluorescens* reduce the severity of rust and leaf spot under field conditions, it is not technically feasible due to increased dosage and economy realized from the crop. Hence, dosage and frequency of application

has to be standardized based on the crop value, which could be as a reliable and practical approach.

7.6 Fruit spray

Pseudomonas syringae (10% wettable powder) in the modified packing line was sprayed at the rate of 10 g/l over apple fruit to control blue and grey mold of apple. The population of antagonist increased in the wounds more than 10 fold during 3 months in storage (Janisiewicz and Jeffers, 1997). Research on the exploration of PGPR have to go a long way to explore its usage to manage post harvest diseases.

7.7 Hive insert

Honey bees and bumble bees serve as a vector for the dispersal of biocontrol agents for the control of diseases of flowering and fruit crops (Sandhu and Waraich, 1985, Kevan *et al*., 2003). An innovative method of application of bio-control agent right in the infection court at the exact time of susceptibility was developed by Thomson *et al*. (1992). A dispenser is attached to the hive and loaded with powder formulation of the PGPR or with other desired biocontrol agent. The foragers when exit the hive, the antagonist get dusted on to bee and delivered to the desired crop, while attempting for sucking the nectar. *Erwinia amylovora* causing fire blight of apple infects through flower and develops extensively on stigma. Colonisation by antagonist at the critical juncture is necessary to prevent flower infection. Since flowers do not open simultaneously the bio-control agent *P. fluorescens* has to be applied to flowers repeatedly to protect the stigma. Nectar seeking insects like *Aphis mellifera* can be used to deliver *P. fluorescens* to stigma. Bees deposit the bacteria on the flowers soon after opening due to their foraging habits. Honey bees have also been used for the management of gray mold of strawberry and raspberry (Peng *et al*., 1992; Sutton, 1995; Kovach *et al*., 2000).

7.8 Sucker treatment

Plant growth promoting rhizobacteria also play a vital role in the management of soilborne diseases of vegetatively propagated crops. The delivery of PGPR varies depending upon the crop. In crops like sugarcane and banana rhizobacteria are delivered through sett treatment or rhizome treatment respectively. Banana suckers were dipped in talc based *P. fluorescens* suspension (500g of the product in 50 liters of water) for 10 min after pairing and pralinage. Subsequently it was followed by capsule

application (50 mg of *P. fluorescens* per capsule) on third and fifth month after planting. It resulted in 80.6 per cent reduction in panama wilt of banana compared to control (Raguchander *et al*., 2000).

7.9 Sett treatment

Red rot of sugarcane is a major production constraint in sugarcane cultivation. Usage of chemical fungicides for the management of red rot was less effective to protect the crop. Since, PGPR act as a predominant prokaryote in the rhizosphere, fluorescent pseudomonads were explored for the management of red rot under field conditions. Viswanathan and Samiyappan (2002) delivered fluorescent pseudomonads through sett treatment. Two budded sugarcane setts were soaked in talc formulation of *P. fluorescens* (20g/l) for one hour and incubated for 18h prior to planting. Planting of treated setts increased cane growth, sugar recovery and reduced red rot incidence under field conditions.

7.10 Multiple delivery systems

Plant pathogens establish host parasite relationships by entering through infection court such as spermosphere, rhizosphere and phyllosphere. Hence, protection of sites vulnerable for the entry and infection of pathogens would offer a better means for disease management. Seed treatment of pigeonpea with talc based formulation of fluorescent pseudomonads at the rate of 4g/kg of seed followed by soil application at the rate of 2.5 kg/ha at 0, 30, and 60 days after sowing controlled pigeonpea wilt incidence under field conditions. The additional soil application of talc based formulation improved disease control and increased yield compared to seed treatment alone (Vidhyasekaran *et al*., 1997). Delivering of *P. fluorescens* as seed treatment followed by three foliar applications suppressed rice blast under field conditions (Krishnamurthy and Gnanamanickam, 1998). Combined application of talc based formulation of fluorescent pseudomonads comprising of Pf1 and FP7 through seed treatment, seedling dip, soil application and foliar spray suppressed rice sheath blight and increased plant growth better than application of the same strain mixture either through seed, seedling dip or soil (Nandakumar *et al*., 2001). Application of strain mixture based formulation of Pf1 and FP7 with or without chitin through seed, seedling dip and foliar spray suppressed leaf folder damage and sheath blight in rice under field conditions (Radja Commare *et al*., 2002). Seed and foliar application of talc based fluorescent pseudomonas reduced leaf spot and rust of groundnut under field conditions (Meena *et al*., 2002). The increased efficacy of strain mixtures through combined application might be due to

increase in the population of fluorescent pseudomonads in both rhizosphere and phyllosphere (Viswanathan and Samiyappan, 1999). Delivering of rhizobacteria through combined application of different delivery systems will increase the population load of rhizobacteria and thereby might suppress the pathogenic propagules.

8 EFFICACY OF FORMULATIONS AGAINST PLANT DISEASES

Plant diseases are in association with crop plants since agriculture began and was managed through synthetic pesticides to increase food production. But continuous usage of pesticides has resulted in the outbreak of pathogens resistant to fungicides apart from environmental pollution. Introduction of PGPR for increasing plant growth promotion during 1950s from the research findings in Soviet Union and in Western countries (Backman *et al*., 1997) opened new vistas to use PGPR as an alternate to chemical pesticides for the management of soilborne pathogens (Dunleavy, 1955; Kloepper, 1993). Application of PGPR either as single strain or strain mixtures based formulations checked pest and disease spread besides increasing growth and yield (Table 2).

8.1 Individual strain based formulations

Plant growth promoting rhizobacteria has diverse applications for the management of plant diseases in agriculture, horticulture and forestry. In addition it also plays a vital role in environmental remediation (Lucy *et al*., 2004). Fluorescent pseudomonads were first developed as talc based formulation for the treatment of potato seed tubers for growth promotion (Kloepper and Scroth, 1981). Treatment of chickpea seeds with *P. fluorescens* (Pf1) through seed followed by root zone application after 30 days of sowing increased seedling emergence, reduced Fusarial wilt incidence caused by *Fusarium oxysporum* f.sp. *ciceris* and increased the yield under field conditions. In addition it also increased the population of Pf1 strain in the rhizosphere (Vidhyasekaran and Muthamilan, 1995). Talc based formulation of *P. fluorescens* strain Pf1 and Pf2 increased grain yield of pigeonpea besides the control of pigeonpea wilt (Vidhyasekaran *et al*., 1997). Seed treatment of groundnut and pigeonpea with peat based formulation of *B. subtilis* supplemented with 0.5% chitin or with 0.5% of sterilized *Aspergillus* mycelium controlled crown rot and wilt of groundnut and pigeon pea respectively. It also increased growth promotion even in the presence of inoculum pressure (Manjula and Podile, 2001). Chitin

supplementation enhances the biocontrol efficacy of formulations. But incorporation of chitin will increase the production cost of biopesticides. Hence, identification of cheap and easy available source of chitin is essential. Seed treatment and soil application of *P. aeruginosa* strain 78 reduced root knot incidence of mungbean besides the reduction in the population density of *Meloidogyne javanica* under field conditions (Ali *et al*., 2002). Seed treatment with wettable powder formulation of *P. putida* strain 30 and 180 suppressed wilt of musk melon to the extent of 63 and 50% after 90 days of transplanting muskmelon in the field. But seed treatments with strain mixtures were not as effective as that of individual strains (Bora, 2004). The decrease in efficacy might be due to the incompatibility of the isolates, which might suppress the genetic expression of defense genes in either bacterial strain.

8.2 Strain mixtures based formulations

Several research outcomes on formulations explain that a single biocontrol agent has the ability to combat a plant pathogen. But, usage of single biocontrol agent in disease management might be also responsible for its inconsistent performance under field conditions. A single biocontrol agent may not perform well at all times in all kinds of soil environment to suppress plant pathogens (Raupach and Kloepper, 1998). In addition application of single biocontrol agent based formulation might have resulted in inadequate colonization, inability to tolerate the extremes of soil pH, moisture and temperature and fluctuations in the production of antimicrobial substances (Weller and Thomashow, 1994). Inconsistent performance of biocontrol agents was overcome by the combined application of several biocontrol strains that mimic the natural environment (Schisler *et al*., 1997; Raupach and Kloepper, 1998). Development of cocktail formulation with compatible isolates will improve disease control through synergy in cross talk between the isolates that lead to increased production of antibiotics at the site of colonization and thereby could suppress the establishment of pathogenic microbes. Advantages of strain mixtures include, broad spectrum of action, enhanced efficacy, reliability and also allow combination of various traits without genetic engineering (Janisiewicz, 1996). Application of mixed PGPR strains based formulations to field might ensure at least one of the mechanism to operate under variable environment that exist under field conditions (Duffy *et al*., 1996).

Application of talc based strain mixture formulation of fluorescent pseudomonads through seed, root, soil and foliage to rice crop suppressed sheath blight under field conditions better than individual strains based formulations. The average disease reduction for mixtures was 45.1% compared to 29.2% for individual strains. In addition to disease reduction

strain mixtures increased biomatter production and yield compared to individual strains (Nandakumar *et al*., 2001). Combined application of *Pichia guilermondii* and *Bacillus mycoides* (B16) reduced the infection of *Botrytis cinerea* by 75% on fruits in strawberry plants grown commercially under greenhouse conditions. But the individual application of either antagonist resulted in 50% reduction of strawberry fruit infection. Population of yeast increased when applied as mixture rather than single application (Guetsky *et al*., 2002). Delivering of talc based strain mixtures of *P. fluorescens* strains (Pf1 and FP7) through seed, soil and foliar reduced sheath blight and leaf folder incidence in rice under greenhouse and field conditions. It also reduced the feeding behavior of leaffolder, reduced larval and pupal weight, and increased larval mortality. Besides, population of parasitoids and spiders also increased in PGPR treated plots (Radja Commare *et al*., 2002).

Table 2.Efficacy of PGPR formulations against plant disease and growth promotion.

Formulation	**Crop**	**Results**	**Reference**
Talc based *P. fluorescens*	Potato	Significant plant growth promotion.	Kloepper and Scroth (1981)
Talc based *P. fluorescens*	Winter wheat	Significant plant growth promotion.	De Freitas and Germida(1992)
Peat based *P. fluorescens*	Cotton	Significant reduction of cotton seedling diseases.	Hagedorn *et al*. (1993)
Talc based *P. fluorescens*	Chickpea	Significant increase in grain yields and controlled fusarial wilt under field conditions.	Vidhyasekaran and Muthamilan, (1995)
Talc based *P. fluorescens*	Pigeonpea	Control of pigeonpea wilt and significant increase in grain yield.	Vidhyasekaran *et al*. (1997)
Chitosan based *B. pumilus*	Tomato	Induced resistance against *F. oxysporum*.	Benhamou *et al.*(1998)
Methyl cellulose and talc based *P. fluorescens*.	Rice	Suppressed rice blast both in nursery and field conditions.	Krishnamurthy and Gnanamanickm (1998)
B. subtilis strain LS213(commercial product)	Watermelon and muskmelon	Increased plant growth, and improved yield.	Vavrina (1999)

Continued table 2.			
B. subtilis Formulations	Cucumber, Watermelon, squash, ornamentals, vegetables, pepper, tobacco, loblolly pine and lodge pine.	Significant induction of resistance against various different pathogens.	Reddy *et al*. (1999); Kenney *et al*. (1999); Martinez- Ochoa *et al*. (1999) ; Ryu *et al*.(1999) ;Yan *et al*. (1999) and Zhang *et al* (1999).
Chitosan based *B. subtilis* strain LS213 (commercial product)	Tomato, tobacco, cucumber and pepper	Reduced the incidence of bacterial spot and late blight of tomato, angular leaf spot of cucumber and blue mold of tobacco.	Reddy *et al*. (1999)
Talc based formulation of *P. fluorescens* (CHAO and Pf1)	Sugarcane	Increased germination of sugarcane seeds, plant growth besides the supper-ssion of damping- off.	Viswanathan and Samiyappan (1999)
Vermiculite based *P. fluorescens*	Sugarbeet	Significant control of damping off	Moenne-Loccoz *et al*. (1999)
Talc based *P. fluorescens*	Rice	Significant reduction of sheath blight under field conditions.	Vidhyasekaran and Muthamilan (1999); Nandakumar *et al*. (2000).
Talc based *P. fluorescens*	Banana	Significant reduction of panama wilt of banana	Raguchander *et al*. (2000)
Vermiculite and Kaolin based *B. subtilis*	Lettuce	Suppressed root rot of lettuce caused by *P. aphanidermatum* and increased fresh weight of lettuce.	Amer and Utkhede (2000)
Vermiculite based *P. putida*	Cucumber	Significantly reduced root rot caused by *Fusarium oxysporum f. sp. cucurbitacearum*	Amer and Utkhede (2000)
Talc based *P. fluorescens* (Pf1)	Urdbean and Sesame	Increased growth promotion and reduced root rot caused by *M. phaseolina*.	Jayashree *et al*.(2000)
Talc based rhizobacterial mixtures of fluorescent pseudomonads	Rice	Significant plant growth promotion and suppression of rice sheath blight.	Nandakumar *et al*. (2001)
Peat based *B. subtilis* supplemented with chitin	Groundnut and pigeon pea	Significant control of groundnut root rot and pigeon pea wilt.	Manjula and Podile (2001)

Continued table 2.			
Chitosan based mixed formulation of *Paenobacillus macerans* and *B. subtilis* (LS255)	Rice	Increased plant growth and yield in rice cultivars, IR24, IR50 and Jyothi.	Vasudevan *et al*. (2002)
Chitin based formulation of *B. subtilis* strain GB03+*B. pumilus* strain INR7(LS256) and *B. subtilis* strain GB03+*B. subtilis* strain IN937b	Tomato and Pepper	Increased yield of pepper and tomato.	Burelle *et al*. (2002)
Talc based *P. aeruginosa* strain 78	Mung bean	Reduced the incidence of root knot and population density of *Meloidogyne javanica* under field conditions.	Ali *et al*. (2002)
Talc based fluorescent Pseudomonads	Sugarcane	Significant increase in sett germination, increased cane growth and reduced red rot incidence.	Viswanathan and Samiyappan (2002)
Talc based *P. fluorescens*	Rice	Significant reduction of rice sheath blight, leaf folder and increased yield. Beside it also increased the population of insect parasites and predators.	Radja Commare *et al*. (2002)
Talc based *P. fluorescens*	Groundnut	Significant reduction of leaf spot and rust of groundnut.	Meena *et al*. (2002)
Talc based formulation of *B. subtilis* and *P. chlororaphis* (PA23)	Tomato	Increased growth promotion and significant reduction of damping off.	Kavitha *et al*. (2003)
Chitosan based mixed formulation of *B. subtilis* strain GB03+*B. pumilus* strain INR7(LS256) and *B. subtilis* strain GB03+*B. pumilus* strain T4(LS257)	Pearl millet	Reduced downy mildew and increased plant growth promotion in pearl millet.	Niranjan Raj *et al*. (2003)
Talc based *P. fluorescens* FP7 supplemented with chitin.	Mango	Significant reduction of anthracnose coupled with increase in fruit yield and quality.	Vivekananthan *et al*.(2004).

Continued table 2.			
Talc based *B. subtilis* (BSCBE4) and *P.chlororaphis* (PA23)	Turmeric	Significant reduction of rhizome rot and yield increase of rhizomes.	Nakkeeran *et al*. (2004)
Talc based *B. subtilis* (BSCBE4), *P. chlororaphis* (PA23) and *P. fluorescens* (ENPF1)	*Phyllanthus amarus*	Significant reduction of stem blight caused by *Corynespora cassicola* under field conditions.	Mathiyazhagan *et al*. (2004)
Talc based *P. putida*	Muskmelon	Effective control of wilt caused by *Fusarium oxysporum* f. sp. *melonis*.	Bora *et al*., (2004)

9 COMMERCIAL PRODUCTS

Research inventions from China, Russia and several other western countries during the early 1950 have proved the potential use of bacteria to be explored for plant disease management (reviewed by Backman *et al*., 1997). Owing to the potential of PGPR, the first commercial product of *B. subtilis* was introduced during 1985 for the use of growers by Gustafson,Inc. (Plano, Texas) in US (Broadbent,s *et al*., 1977). The strains of *B. subtilis* A-13, GB03, GB07 were sold for the management of soilborne pathogens under the trade names of Quantum@, Kodiak@ and Epic@ respectively (Broadbent,s *et al*., 1977). Release of *Bacillus* based products during 1985 has resulted in the increase in market size for the usage of bacterial products in crop disease management. Backman *et al*. (1977) stated that 60-75% of the cotton crop in US is treated with *B. subtilis* for the management of soilborne pathogens encountered in cotton ecosystem. Among several PGPR strains *Bacillus* based products gains momentum for commercialization. Because, *Bacillus* spp., produce endospores tolerant to extremes of abiotic environments such as temperature, pH, pesticides and fertilizers (Backman *et al*., 1997). Owing to the potentiality of *Bacillus* spp., 18 different commercial products of *Bacillus* origin are sold in China to mitigate soilborne diseases (Backman *et al*., 1997). The registered commercial products of PGPR are listed in Table 3. Details of registered products are in the web sites:
http://www.ippc.orst.edu/biocontrol/biopesticides/;
http://www.epa.gov/pesticides/biopesticides/.

10 IMPROVEMENT OF FORMULATION EFFICACY

In general, though biocontrol agents perform well in the management of plant diseases, they are highly sensitive to the fluctuations in environmental conditions and are inconsistent in their performance. The consistency of biocontrol agents could be enhanced through several means without going in for genetic engineering. Since nature is bestowed with millions of beneficial microbes, development of compatible cocktail of beneficial microbes would increase the efficiency of their performance. Strategies to enhance the efficacy of biocontrol organisms include

1. Development of compatible consortia.
2. Strains that induce synergistic expression of biocontrol genes.
3. Adjuvants, spreaders and stickers.
4. Genetic engineering of PGPR strains.
5. Formulations comprising of compatible PGPR strains and plant inducers of chemical origin.

Table 3. Commercial products of PGPR in plant disease management

Product	**Target pathogens/diseases**	**Crops recommended**	**Manufacturer**
Bio-Save 10, 11, 100, 110, 1000 TM – *P. syringae ESC-100*	*Botrytis cinerea, Penicillium spp, Mucor pyroformis, Geotrichum candidum*	Pome fruit (Biosave 100) and Citrus (Biosave 1000)	Eco Science Corp, Produce Systems Div., Orlando
Blight Ban A506 – *P. fluorescens* A 506	*Erwinia amylovora* and russet - inducing bacteria	Almond, Apple, Apricot, Blueberry, Cherry, Peach, Pear, Potato, Strawberry,Tomato	Plant Health Technologies, USA
Cedomon TM – *P. chloroaphis*	leaf stripe, net blotch, *Fusarium sp,* spot blotch, leaf spot and others	Barley and Oats, potential for wheat and other cereals	Bio Agri AB, Sweden
Campanion – *B. subtilis* GB03	*Rhizoctonia, Pythium, Fusarium and Phytophthora*	Horticultural crops and turf	Growth products, USA
Conquer TM - *P. fluorescens*	*P. tolassii*	Mushrooms	Mauri Foods, Australia

Continued table 3.

Victus™ – *P. fluorescens*	*P. tolassii*	Mushrooms	Mauri Foods, Australia
BioJect Spot – less – *P. aureofaciens*	Dollar spot, Anthracnose and *P. aphanidermatum*	Turf and other crops	Eco Soil Systems, San Diego, CA
BioJet™ – *Pseudomonas* sp + *Azospirillum*	Brown batch and Dollar spot disease	Turf and other crops	Eco Soil Systems, San Diego, CA
Deny - *Burkholderia cepacia (Pseudomonas cepacia*)	*Rhizoctonia, Pythium, Fusarium* and diseases caused by lesion, spiral, lance, and sting nematodes.	Alfalfa, Barley, Beans, Clover, Cotton, Peas, Sorghum, Vegetable crops and Wheat	Stine Microbial Products, Shawnee, KS
Intercept ™- *P. cepacia*	*Rhizoctonia solani, Fusarium* sp., *Pythium sp.*	Maize, Vegetables, Cotton	Soil Technologies Corp, USA
Kodiak™, Kodiak HB™, Epic™, Concentrate ™, Quantum 4000 and System 3 ™ – *B. subtilis* GB03	*Rhizoctonia solani, Fusarium spp, Alternaria spp, and Aspergillus spp*	Cotton, Legumes	Gustafson, Inc., Dallas, USA
Bio Yield – Combination of *B. subtilis* and *B.amyloliquefaciens*	Broad spectrum action against greenhouse pathogens	Tomato, Cucumber, Pepper and Tobacco	Gustafson, Inc., Dallas, USA
Rhizo-Plus – *B. subtilis* strain FZB24	Against *R. solani, Fusarium spp., Alternaria spp., Sclerotinia* and *Verticillium.*	Greenhouses grown crops,forest tree seedlings, ornamentals, and shrubs.	KFZB Biotechnik GMBH, Berlin, Germany.
Serenade – *B. subtilis* strain QWT713. Available as wettable powder.	Powdery mildew, Downy mildew, Cercospora leaf spot, early blight, late blight, brown rot, fire blight and others.	Cucurbits, Grapes, Hops, Vegetables, Peanuts, Pome fruits, stone fruits and others	AgraQuest, Inc., Davis, USA.

Continued table 3.			
Rhapsody – *B. subtilis* strain QST713. Aqueous suspension formulation	Powdery mildew, sour rot, downy mildew, and early leaf spot, early blight, late blight, bacterial spot, and walnut blight diseases.	Cherries, cucurbits, grapes, leafy vegetables,peppers, potatoes, tomatoes, and walnuts.	AgraQuest, Inc., Davis, USA.
Subtilex - *B. subtilis* MB1600	*Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp.	Ornamental and vegetable crops	Becker Underwood, Ames.
GB 34 Concentrate Biological Fungicide - *B. pumilus*	*Rhizoctonia* and *Fusarium*, which attack developing soybean roots	Soybean	Gustafson LLC1400 Preston Road TX 75093
SonataTM ASO *B. pumilus* strain QST 2808	Fungal pests such as molds, mildews, blights, rusts and to control Oak death syndrome	Used in nurseries, landscapes, oak trees and green house crops	Agra Quest, Inc.,Davis, USA
System 3 - *Bacillus subtilis GB03* and chemical pesticides	Seedling pathogen	Barley, Beans, Cotton, Peanut, Pea, Rice, Soybean	Helena Chemical Co.,Memphis USA
AtEze *P. chlororaphis* strain 63-28	*Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum*	Ornamentals and vegetables	EcoSoil Systems, Inc., San Diego, CA
Pix plus plant regulator, *B. cereus* BPO1 technical, - *B. cereus* strain UW85	Used as growth regulator	Cotton	Micro Flo Company, Lakeland, FL 33807
Bio-save 10LP, 110 – *P. syringae*	*Botrytis cinerea*, *Penicillium* spp., *Geotrichum candidum*	Pome fruit, Citrus, Cherries and Potatoes	Eco Science Corp., FL 32779.

10.1 Development of compatible consortia

Biological control of plant pathogens in disease suppressive soil is due to the existence of mixture of microbial antagonists (Lemanceau and Alabouvette, 1991). Hence, augmentation of compatible strain mixtures of PGPR strains to infection court will mimic the natural environment and

could broaden the spectrum of biocontrol against different plant pathogens (Janisiewicz, 1988). Efficiency of biocontrol agents could be increased by the development of compatible strain mixtures of different biocontrol organisms by considering the following norms (Raupach and Kloepper, 1998).

1. Strain mixtures that differ in the pattern of plant colonization
2. Strain mixtures with broad spectrum of action against different plant pathogens
3. Strain mixtures with different modes of action
4. Strain mixtures with genetically different organisms having the capability to perform in different pH, moisture, temperature and relative humidity.

Vidhyasekaran and Muthamilan (1995) found that *P. fluorescens* - Pf1 was not inhibitory to nitrogen fixing bacteria, *Rhizobium* and *Azospirillum*. Development of strain mixtures with non-competitive nature of these bacterial strains will have an additive effect in increasing the yield and growth. Strain mixtures of Pseudomonads in combinations with other bacteria were found effective than the application of individual organisms (Duijff *et al*., 1999). Application of the mixture of phloroglucinol producers of *P. fluorescens* F113 and a proteolytic rhizobacterium suppressed sugar beet damping-off (Dunne *et al*., 1998). Combination of iron chelating Pseudomonas strains and inducers of systemic resistance suppressed Fusarium wilt of radish better than the application of individual strains (de Boer *et al*., 2003).

10.2 Strains that induce synergistic expression of biocontrol genes

Development of products with strains that induce the expression of biocontrol genes can also increase the bioefficacy of PGPR strains under field conditions. Combination of CHAO and Q287 of fluorescent pseudomonads enhanced the expression of the genes that code for diacetyl phloroglucinol. This would lead to the increase of DAPG pool in the rhizosphere and will suppress the disease causing organisms (Raaijmakers *et al*., 1999).

10.3 Adjuvants, spreaders and stickers

In general, the performance of PGPR formulations in controlling plant diseases is inconsistent. Since, disease suppression is the outcome of interactions between biocontrol agents, pathogen, plant and environment,

any fluctuations in growing seasons; environmental conditions and high inoculum pressure alter the efficacy of biocontrol formulations. Integrating the usage of formulations with other management strategies that aims at increasing the productivity of the crop could enhance the efficacy of formulations (Larkin *et al*., 1998). Performance of biocontrol agents in the formulations can be increased by the incorporation of water-soluble adjuvants, oils, stickers and emulsions. It increases the efficacy of biocontrol agents by supplying nutrients and by protecting the microbes from desiccation and death (Connick *et al*., 1991; Bateman *et al*., 1993; Barnes and Moore, 1997; Green *et al*., 1998; Ibrahim *et al*., 1999). Incorporation of carboxy methyl cellulose (CMC) in formulations serves as stickers in uniform seed coating of microbes. Though adjuvants and stickers increase the efficacy of bio-products it has its own demerits. Adjuvants/stickers in the formulations will be diluted when exposed to rain or heavy dew. It would alter the efficacy of formulations by reducing the establishment or colonization of PGPR onto the infection court. Sometimes spray application of emulsions or oil-based formulations may be toxic to plants. Hence, a thorough knowledge on the usage of adjuvants, stickers is essential for increasing the efficacy of formulations.

10.4 Genetic engineering of PGPR strains

Genomic tinkering of naturally occurring PGPR strains with genes that are beneficial to plants will lead to the accentuated expression of the genomic products which could alleviate the attack of both pests and diseases. This will facilitate for the introduction of a single bacterium with multiple modes of action to benefit the growers to save their crop with increased returns by reducing the inputs invested for plant protection measures. However, the release of genetically modified organisms is a policy decision to be made by the policy makers. Hence, appraising of policy makers about the safe usage of beneficial bacteria will be a boon to the farming community and environment.

10.5 Formulations of PGPR strains compatible with plant inducers of chemical origin

Plant inducers of chemical origin are used to trigger systemic resistance at very low concentrations against pests and diseases. The chemical inducer benzothiadiazole (Bion) is commercially used for inducing resistance in crop plants against pests and diseases (Gorlach *et al*., 1996). Hence, identification of PGPR strains that has compatibility with chemical inducers will have a synergistic action against pests and diseases.

11 COMMERCIALIZATION

Industrialization of biocontrol agents requires linkage between corporate and academic bodies. The success and commercialization of a scientific innovation depends on the availability of the technology to the end users. It depends on the linkages between the scientific organization and industries. Biocontrol technology could become as a successful component of plant protection only when it is commercialized.

11.1 Stages of commercialization

Stages of commercialization include isolation of antagonist, screening, pot test and field efficacy, mass production and formulation development, fermentation methods, formulation viability, toxicology, industrial linkages and quality control (Sabitha Doraiswamy *et al*., 2001).

11.1.1 Isolation of antagonist

Isolation of an effective strain plays a prime role in disease management. It is done from the pathogen suppressive soils either by dilution plate technique or by baiting the soil with fungal structures like sclerotia of pathogen. Consortium of biocontrol agents could be established by isolating the location specific and crop specific isolates. It could be used for the development of mixtures of biocontrol agents suited for different ecological niche.

11.1.2 Screening of antagonist

All the strains isolated from the different cropping system have to be ascertained for its virulence and broad spectrum of action against different pathogens causing serious economic threat to cultivation. Selection of an effective strain decides the viability of the technology. Hence a proper yardstick should be developed to screen the antagonistic potentiality of the biocontrol agents. *In vitro* screening of the antagonist through dual culture technique alone could not be an effective method for strain selection. To be an effective antagonist it should possess a high level of competitive saprophytic ability, antibiosis, should have the ability to secrete increased level of cell wall lytic enzymes (chitinases, glucanases and proteases), antibiotics and plant growth promotion. Hence the yardstick should be developed, comprising of above-mentioned components. Each component should be given weightage depending upon their role in disease management. This type of rigorous and meticulous screening will lead to

identification of an effective biocontrol strain suited for commercialization. Twenty rhizobacterial isolates from strawberry rhizosphere were evaluated for its antifungal action against *Verticillium dahliae*. The selection of best antagonistic bacterial isolate was done by screening for the antifungal action against different soilborne pathogens apart from the target pathogen. In addition it was also tested for the antifungal mechanism of the rhizobacteria for the production of lytic enzymes (chitinases, glucanases and proteases) and plant growth promotion. Collectively all these parameters were combined based on bonitur scale (28 points). The strain that had the highest score was selected for testing its efficacy under greenhouse. Among twenty strains, *P. putida* E2 had the maximum bonitur scale of 28 points and was highly effective in suppressing *Verticillium* wilt of strawberry under greenhouse conditions. It was found to perform better than the commercial product Rhizovit (Berg *et al*., 2001). This clearly explains that selection of bacterial antagonist plays a major role in commercialization of the bacteria for disease management. Initial mistake committed in strain selection will lead to complete failure of the technology.

11.1.3 Pot test and field efficacy

The plant, pathogen and antagonists are co exposed to controlled environmental conditions. Exposure of the host to the heavy inoculum pressure of the pathogen along with the antagonist will provide ecological data on the performance of the antagonist under controlled conditions. Promising antagonists from controlled environment are tested for its efficacy under field conditions along with the standard recommended fungicides. Since the variation in the environment under field condition influence the performance of biocontrol agent, trials on the field efficacy should be conducted for at least 15 – 20 locations under different environmental conditions to promote the best candidate for mass multiplication and formulation development (Jeyarajan and Nakkeeran, 2000).

11.1.4 Mass production and formulation development

The first major concern in commercial production systems involves the achievement of adequate growth of the biocontrol agent. In many cases biomass production of the antagonist is difficult due to the specific requirement of nutritional and environmental conditions for the growth of organism. Mass production is achieved through liquid and semisolid and solid fermentation techniques. The commercial success of biocontrol agents requires

- Economical and viable market demand
- Consistent and broad spectrum action

- Safety and stability
- Longer shelf life
- Low capital costs
- Easy availability of career materials (Jeyarajan and Nakkeeran, 2000)

11.1.5 Fermentation

Liquid and solid fermentation methods are used for the mass production of PGPR.

11.1.5.1 Liquid fermentation

This fermentation system has been adopted for the mass multiplication of fungal bacterial biocontrol agents. For mass multiplication the selected medium should be inexpensive and readily available with appropriate nutrient balance. Kings' B broth or nutrient broths are used for the mass production of *Pseudomonas* and *Bacillus* spp., through liquid fermentation technology (Kloepper and Schroth, 1981; Vidhyasekaran and Muthamilan, 1995; Manjula and Podile, 2001; Nakkeran *et al*., 2004).

11.1.5.2 Solid fermentation

In nature wide range of organic substrates could be used for the solid-state fermentation for mass multiplication. Solid fermentation media consisting of inert carriers with food bases was used for mass production of biocontrol agents (Lewis, 1991).The media with relatively low microbial content would be suited for solid-state fermentation and for the amendment of biocontrol agents. Solid substrates include straws, wheat bran, sawdust, moistened bagasse, sorghum grains, paddy chaff, and decomposed coir pith, farmyard manure and other substrates rich in cellulose for inoculum production. Siddiqui and Mahmood (1999) stated that bacteria had great potential to manage plant parasitic nematodes. But the practicality of the same could be done by incorporating the antagonistic bacteria to organic manures, followed by incubation at 35°C for 5-10 days coupled with frequent mixing under sterile environment along with water so as to maintain the organic manure under moist conditions, which aid in the proliferation of the bacteria. The enriched organic manure with biocidal value could be used for the management of nematodes and plant growth promotion.

11.1.6 Formulation viability

Shelf life of the formulations decides the commercialization of biocontrol agents. Formulations should support the viable nature of the product for the increased period of storage. Bio control product should have the minimum shelf life of 8-12 months for industrialization. Carrier material should not affect the viable nature of the biocontrol agent. Commercialization of the bioproducts is mainly hampered due to the poor shelf life. Hence research should be concentrated to increase the shelf life of the formulation by developing superior strains that support the increased shelf life, or the organic formulations that support the maximum shelf life with low level of contaminants must be standardized for making biocontrol as a commercial venture.

11.1.7 Toxicology

Safety and environmental considerations could not be taken for granted and it is crucial that biopesticides are regulated in an appropriate way to confirm the international standards. The regulatory environment is generally favorable for the bio-pesticides than the chemical pesticides. However the cost of carrying out the toxicological study for registration is still prohibitive. Toxicology includes information of antagonist on the safety to men, plants, animals and soil microflora. Cost incurred for the toxicological studies is high. These studies have to be done separately for each and every biocontrol organism separately. The huge investment on the toxicological studies warrants for the linkages between stakeholders and research organizations (Jeyarajan and Nakkeeran, 2000; Sabitha Doraiswamy *et al*., 2001).

11.1.8 Industrial linkages

The research institutes carry out the initial discovery of an effective organism, genetic manipulation of organisms to develop superior strains, and studies on mechanisms, field efficacy and protocols for the development of formulations. But to take this technology to entire country depends on the partnership between the stakeholders and institutes. Corporate resources are required for the large-scale production, toxicology, wide scale field-testing, registration and marketing. Entrepreneurship may be defined as the exchange of intellectual property for research grants, and a royalty stream, with the establishment of University – Industry partnership for the benefit of both. The first requirement for the entrepreneurship requires a patent application on the strain and the related technology, especially on the efficacy data, identity of the organism, toxicological data and delivery

system. Ideally the process of entrepreneurship will result in an academic corporate research team working towards a common goal.

11.1.9 Quality control

This is very much required to retain the confidence of the farmers on the efficacy of biocontrol agents. Being the living organisms their population in a product influences the shelf life. The population load of the antagonists decides the minimum level of requirement for bringing the effective biological control of the plant diseases. Depending on the type of the antagonist (bacteria or fungal), and formulation, the moisture content and population load varies. The other contaminating organisms should be also under the permissible limits.

12 CONSTRAINTS TO COMMERCIALIZATION

The success of microbial pesticides to suppress pests and diseases depends on the availability of microbes as a product or formulation, which facilitate the technology to transfer from lab to land. The constraints to biopesticides development and utilization mirror some of those factors that limit the development worldwide. Constraints include

- Lack of suitable screening protocol for the selection of promising candidate of PGPR.
- Lack of sufficient knowledge on the microbial ecology of PGPR strains and plant pathogens
- Optimization of fermentation technology and mass production of PGPR strains
- Inconsistent performance and poor shelf life
- Lack of patent protection
- Prohibitive registration cost (Schisler and Slininger, 1997; Fravel *et al*., 1998; Fravel *et al*., 1999)
- Awareness, training and education shortfalls
- Lack of multi disciplinary approach
- Technology constraints (Sabitha Doraiswamy *et al*. 2001).

12.1 Screening and selection of potential PGPR strain

Success of commercialization of PGPR strains depends on the selection of effective strains after adopting rigorous screening strategies. Because, any mistake during strain selection will be a costly mistake in product development (Schisler and Slininger**,** 1997). The potentiality of the

PGPR strain in the suppression of plant pathogen should be carried out at both lab and field conditions in different soil types with diversified microbial communities and climatic conditions (Roberts and Lohrke, 2003). It would lead to the development of a viable PGPR strain.

12.2 Microbial ecology and interaction

Suppression of plant disease is a four-way interaction of biocontrol agents, plants, pathogens and the environment. Hence, understanding of interaction between all these components is essential for developing a suitable biocontrol agent in disease management (Handelsman and Stabb, 1996; Larkin *et al*., 1998). Extracellular metabolites produced by PGPR strains interact with microbial community (plant pathogens and other microbes) and plant in rhizosphere or spermosphere or phyllosphere and result in the suppression of pathogenic propagules either by direct action of antibiotics or through elicitation of induced systemic resistance activated by the molecular determinants (lipopolysaccharide, salicylic acid), global regulators and siderophores of bacterial origin (Larkin *et al*., 1998; Thomashow *et al*., 1990; Thomashow and Weller, 1988; Loper and Henkels, 1999**).** However, knowledge on the influence of biotic and abiotic environment on PGPR strains to express its antimicrobial action has to be studied in depth under *in vivo* to improve the efficacy of PGPR strains. This will facilitate to identify bacterial strains that could perform well under diverse environmental conditions around the court of infection.

12.3 Fermentation technology and shelf life of formulations

Optimization of fermentation technology (Liquid or solid fermentation) with suitable medium (synthetic or semi-synthetic) for mass multiplication and identification of suitable carrier material (organic or inorganic) for formulation development with increased shelf life is a barrier in the commercial success of formulation development. Slininger *et al***.** (1996) reported that liquid culture and formulation technologies has to be optimized for the commercial exploitation of *P. fluorescens* 2-79 for the management of take all disease of wheat. Commercial biomass production of bacterial antagonists requires large-scale fermenters. The biomass production and efficacy of biocontrol agents to suppress plant pathogens varies depending on the nutrient composition of the medium (Schisler and Slininger, 1997). Hence, the medium selected for biomass production should support the growth and efficacy of antagonist and the cost of medium should be economical so that the technology remains viable.

12.4 Patent protection and prohibitive registration cost

The environmental protection agency in developed and developing countries should relax the formalities and registration cost to promote registration of biocontrol agents either by universities or private companies. The patent protection rights for the effective products should be strengthened to encourage the organizations involved in identification and development of commercial biocontrol agents.

12.5 Awareness, training and education shortfalls

The general level of awareness among stakeholders about the potential value of biopesticides is lacking. There is a need for

- Awareness level among the policy makers of the potential for biopesticides, their efficacy and their effect in reducing the health and environmental problems.
- The opportunities offered by the commercialization in terms of generation of wealth and employment are to be promoted.
- Entrepreneurs and investors need to be informed about the opportunities that exist for establishing commercial companies to manufacture market and sell biopesticides.
- Government extension workers have to be trained in biopesticides and the communication between research and extension sectors have to be intensified.
- The nature and mode of action of biopesticides have to be explained to farmers who are used to chemical insecticides, which are often fast acting and are visibly effective (Sabitha Doraiswamy *et al*., 2001**).**

12.6 Lack of multidisciplinary approach

The process of biopesticides development to complete product requires research in areas of screening, formulation, field application, production, storage, toxicology as well as the steps necessary for commercialization, such as scale up production, registration and regulatory matters. Most of the research efforts undertaken with the use of biopesticides are confined only to the exploration, collection, isolation and identification of biocontrol agents combined with laboratory based bioassays. But in the process of product development the above research aspects shares only a fraction of work required to develop a complete product. Product development requires a multidisciplinary approach to biopesticides research

and development. Rarely a complete range of expertise exists in a single institute or organization.

12.7 Technology constraints

12.7.1 Delivery system

Success in biocontrol depends on understanding and use of delivery system. The research on delivery system is well below that of chemical insecticides. The attention on the application technology can improve biopesticides performance.

12.7.2 Biopesticides quality

The major problem in the field of biopesticides production is the product quality and stability. In small-scale production, contamination of inoculum is a common problem. The long -term shelf life of the product is highly essential to attract the multinational companies to invest on a large scale.

13 STRATEGIES TO PROMOTE COMMERCIALIZATION

Commercialization of biocontrol could be promoted by

- Popularization of biocontrol agents
- Industrial linkages

13.1 Popularization of biocontrol agents

Motivating the growers through

a. Publicity
b. Field demonstrations
c. Farmers days
d. Biovillage adoption
e. Conducting periodical trainings for commercial producers and farmers to increase / improve the supply.

13.2 Industrial linkages

a. Technical support should be made available to entrepreneurs on quality control and registration.
b. Regular monitoring is essential to maintain the quality.
c. Constant research support should be extended to standardize the dosage, storage, and delivery systems. Positive policy support from Government to use more of biocontrol agents in crop protection.

14 CONCLUSION

Increase in public concern about the environment has increased the need to develop and implement effective biocontrol agents for crop protection. An effective PGPR could be developed for disease control only after understanding its performance in the environment in which it is expected to perform. In nature agriculture crops are exposed to diverse environmental conditions and gambling of monsoons, which alter the microclimatic conditions existing around the infection court. A thorough knowledge on the mechanisms and performance related to disease control will help in the selection of promising candidates that suits industries to produce reliable commercial products (Collins *et al*., 2003).

Introduction of PGPR strains to phyllosphere, spermosphere or rhizosphere may be moderately effective or sometimes totally ineffective under field conditions to control plant diseases (Duffy *et al*., 1996)**.** Inefficacy of the strains under field conditions may be due to the variation in climatic conditions that suppress growth and survival of biocontrol agents (Guetsky *et al*., 2001)**.** In addition both pathogen and biocontrol agents does not have similar ecological niche for their growth and survival. Hence the efficacy of biocontrol agents could be improved through the usage of compatible mixed inoculum of biocontrol agents which could have a consistent performance under diverse environmental conditions (Guetsky *et al*., 2001; Janisiewicz, 1996)

PGPR formulations comprising of bacterial strain mixtures having the capability to induce chitinase in plant play an important role in hydrolyzing chitin, the structural component in gut linings of insects and would lead to better control of insect pest (Broadway *et al*., 1998). In addition certain PGPR strains also activate octadecanoid, shikimate and terpenoid pathways. This in turn alters the volatile production in the host plant leading to the attraction of natural enemies (Bell and Muller, 1993). Identification of entomopathogenic PGPR strains that have the capability to colonize phylloplane in a stable manner will be a breakthrough in the management of foliar pests (Otsu *et al*., 2004). Combined application of

entomopathogenic strains with compatible PGPR strains that have the ability to suppress plant diseases has to be developed for broad spectrum action.

On the contrary, certain studies explain that some strain mixtures perform even lower than that of individual strains. So, the basic knowledge on molecular signaling mechanisms between related strains and species has to be understood for the development of a better formulation that could suppress a broad spectrum of pathogens and pests besides plant growth promotion.

The formalities involved in registration of formulation are very stringent and the cost incurred for registration of individual strains is also high. At this juncture, the cost incurred for the registration of formulations with mixed strains should not be prohibitive to the industrialist to venture in to the field of commercialization of the organism. If it is found to be prohibitive than the research developments from the lab would not reach the end-users.

But one cannot compensate the quality and safety of the product for the use of farming community. The advocates of biocontrol also face a tough time to convince the environmental protection agencies about the safety of the organisms. Because, acceptance, registration, transfer of technology and adoption of the biocontrol agents at field level relies on the safety of the organism to be used. Biocontrol researchers cannot deny that several well known bacterial biocontrol agents have a threat to become as an opportunistic pathogen. Occurrence of immune compromising infectious diseases and tissue transplants has made opportunistic pathogens as a visible threat to human health.

Several potential biocontrol agents used for plant disease management behave as opportunistic human pathogens. Though *P. aeruginosa* is a potential biocontrol agent of gray leaf spot on turf, it is also a virulent opportunistic pathogen which infects wounds and severe burns. *P. cepacia,* which is used for the management of pea root rot, has the capability to infect lungs of the patients having cystic fibrosis. *Bacillus cereus*, being a potential candidate for the management of damping-off and root rot of soybean, it is also a food contaminant and closely mimics *Bacillus anthracis*, the causal agent of anthrax disease. The confusions involved in distinguishing between the related strains that turn as opportunistic pathogens for humans has to be solved to convince the policy makers and environmental protection agencies to promote acceptance, registration, transfer of technology and adoption.

Amidst these obstacles, since PGPR has its own potentiality in plant disease and pest management several products have been registered for the practical use of farming community. Sixty to 75% of cotton crops raised in U.S. are treated with commercial product of *B. subtilis* (Kodiak) effective against soilborne pathogens such as *Fusarium* and *Rhizoctonia*. It is also

used in peanut, soybean, corn, vegetables and small grain crops (Backman *et al*., 1997). In China, PGPR has been in commercial development for over than two decades and are referred as yield increasing bacteria (YIB). It is applied over an area of 20 million hectares of different crop plants (Chen *et al*., 1996; Kilian *et al*., 2000). In India, more than 40 stakeholders from different provinces have registered for mass production of PGPR with Central Insecticide Board, Faridabad, Haryana through collaboration with Tamil Nadu Agricultural University, Coimbatore, India for the technical support and information (Ramakrishnan *et al*., 2001). Though the market size for PGPR usage is increasing constantly under greenhouse and field conditions, finding solutions for the above obstacles will create a break through in the adoption of biocontrol agents for field applications.

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