DISCUSSION
CHAPTER V
DISCUSSION

All plants are hosts to one or more endophytic microorganisms. However the endophyte plant interaction is one of the least studied biochemical systems in nature. Endophytes include the fungi, bacteria, and actinomycetes that primarily reside in the tissues beneath the epidermal cell layers and the host tissues are transiently symptomless and inconspicuous (Stone et al., 2000). Conceivably, the microbes live within the intercellular spaces of the tissues, and it also seems likely that penetration of living cells may occur, but it is not easy to observe. There is ample evidence that many endophytic bacteria have beneficial effects on plants (Hallmann et al., 1997). Growth promotion of plants may be achieved by bacterial production of plant growth regulators such as auxins, cytokinins and gibberellins; nitrogen or other nutrients may be provided by biological nitrogen fixation or mobilized as is the case for phosphorus; moreover, endophytes may confer plant protection against pathogens by induction of plant defense mechanisms, pathogen-antagonistic substances or through competition for colonization sites and nutrients.

The present work is carried out to study the diversity of endophytes of crop plants and to elucidate their role in plant growth promotion.

5.1. Occurrence of endophytic microorganisms in the roots and shoots of crop plants

In the present study the endophytic bacteria were isolated from eight crop plants viz. rice (Oryza sativa), ragi (Eleusine corocana), cowpea (Vigna unguiculata), soybean (Glycine max), groundnut (Arachis hypogea), sunflower (Helianthus annuus), chilli (Capsicum annum) and tomato
*Lycopersicon esculentum*. The samples were collected after 45 days of sowing, before flowering initiated. Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. Bacterial endophytes are found in a variety of plants, such as sugar beet (Dent *et al.*, 2004), prairie plants, soybean, sorghum, wheat and maize crops (Zinniel *et al.*, 2002), potato varieties (Sessitsch *et al.*, 2002), wheat (Germida and Siciliano, 2001), and rice (Sun *et al.*, 2008). The presence of different endophytic species in soybean depends on the plant genotype, the plant age, the tissue sampled, and also on the season of isolation (Kuklinsky-Sobral *et al.* 2004). However not much work has been done on field crops in India.

In the present study, the endophytes in the shoots and roots were isolated by the technique of surface sterilization using sodium hypochlorite and plating in agar media. The sterility check carried out by plating the last wash water confirmed that the bacteria obtained on the plates were endophytes (Gyaneshwar *et al.*, 2001). Samples were discarded if any growth was detected in the sterility check. Hence the bacteria obtained after plating by this procedure was tentatively identified as putative endophytes.

Earlier studies on the diversity of bacterial endophytes also have focused on characterization of isolates obtained from internal tissues following disinfection of plant surfaces with sodium hypochlorite or similar agents (Miche and Balandreau, 2001). Criteria to recognize “true” endophytic bacteria have been published (Reinhold-Hurek and Hurek 1998a). Use of the term putative endophytes has been recommended for those not validated microscopically (Rosenbleuth and Martinez-Romero, 2006).
5.1.1. Enumeration of endophytic bacterial population on shoot and root regions of crop plants

In the present study, the population of endophytic bacteria in the shoot region ranged from $5.8 \times 10^5$ cfu/g in cowpea to $1.3 \times 10^5$ cfu/g in sunflower. The highest population of root endophytes was recorded in the pulse crops, cowpea and soybean (6.5 and $6.1 \times 10^5$ cfu/g) while the least was in the oilseed crops, groundnut and sunflower (3 x $10^5$ and 2.8 x $10^5$ cfu/g). Hung and Annapurna (2004) reported $2.3 \times 10^5$ cfu/g in roots and $3.6 \times 10^5$ cfu/g in stem of soybean. Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). In general, endophytic bacteria occur at lower population densities than rhizospheric bacteria or bacterial pathogens (Hallmann et al. 1997; Rosenblueth and Martinez-Romero, 2004).

The number of endophytes recorded was more in roots than shoots in all the crops studied. The larger endophytic bacterial populations in roots and decrease in the stems and leaves was reported by Lamb et al. (1996). Natural endophyte concentrations can vary between $10^2$ and $10^6$ cfu/g for alfalfa, sweet corn, sugar beet, squash, cotton, and potato, and the population levels were found to be $10^3$ to $10^5$ cfu/g of plant tissue for tomato and potato (Kobayashi and Palumbo, 2000). In the present study the solanaceous vegetables recorded $5.4 \times 10^5$ and $4.6 \times 10^5$ cfu/g in root and $4.2 \times 10^5$ and $3.9 \times 10^5$ cfu/g in shoot. The pulses recorded more number of isolates than all other groups of plants tested. These differences in overall colonization rates are related to climatic conditions (temperate versus tropical) and genotypic compatibility between the host and endophyte.
5.1.2. Characterisation of the endophytic isolates

The diversity of the putative endophytic bacteria isolated from different tissues of the hosts was assessed using phenotypic characterization methods in the present study. Colony morphology gave an indication of the variation among the endophytes. The isolates studied were chosen for their dominance as well as uniqueness or differences with others in colony morphology. Nineteen isolates were selected from the 4 groups of plants and were named according to the host plant.

Seven of these bacterial isolates were Gram positive while twelve were Gram negative. The gram positive bacteria predominated the isolates from pulses and cereals compared to sunflower and vegetables. Earlier workers have reported a predominance of Gram negative bacteria in the tissues of various plants (Stoltzfus et al., 1997). However, Zinniel et al. (2002) reported an equal presence of Gram negative and Gram positive bacteria in soybean, sorghum, wheat and maize.

All the isolates except ragi isolate ECE6 and tomato isolate LEE19 were motile in the present study. Motility is an important characteristic for endophytes. Although endophytic bacteria can follow water fluxes for passive movement, they also need to be able to move inside the plant since endophytes tend to colonize specific plant parts that do not always correspond to the port of entry in the plant (Taghavi et al., 2009).

5.1.3. Tentative identification of endophytic bacterial isolates

Based on the morphological, microscopic and biochemical characters and their pattern of utilization of carbon sources the endophytic bacterial isolates were tentatively identified according to Bergey’s Manual of Determinative Bacteriology. Of the nineteen isolates, seven isolates belonged to the genus Bacillus and three isolates could be identified to the species level and are as follows. The rice isolate OSE1
and the ragi isolate ECE5 were tentatively identified as *Bacillus pasteurii* while cowpea isolate VUE 13 was identified as *Bacillus megaterium*. The Gram negative bacteria belonged to the genera *Citrobacter, Klebsiella, Acinetobacter, Pseudomonas* and *Vibrio* which were predominantly present in vegetables. *Bacillus* and *Pseudomonas* were found in pulses while *Bacillus sp.* dominated the cereal isolates. These are in accordance with the results of earlier workers.

According to Jacobs *et al.* (1985) the most common taxa of endophytic bacteria recovered include *Bacillus, Enterobacter, Erwinia, Pseudomonas* and *Flavobacterium*. Fisher *et al.* (1992) identified endophytes in maize belonging to eight different genera; majority belonging to *Pseudomonads*. McInroy and Kloeppeper (1995) reported that the endophytic bacterial diversity spanned over 40 genera, with predominance of *Pseudomonads* and *Bacillus*. Hallmann *et al.* (1997) have reported that the former Pseudomonas group (*Pseudomonas, Burkholderia*) and Enterobacteriacea (*Enterobacter, Klebsiella*) are the common taxa found in tomato, potato, cotton, soybean, rice and maize. These taxa were predominantly found in the present study.

### 5.2. Enzymatic activities of the endophytic bacterial isolates

The enzymatic activity of the endophytic bacterial isolates was studied in relation to gelatinase, amylase, cellulase and pectinases. All the isolates except the rice isolate OSE2 and sunflower isolates HAE7 and HAE8 were able to produce cellulase. Similar results were reported by Jalgaonwala and Mahajan (2011) (amylase, cellulase and gelatinase activities) in the endophytic isolates of *Pongamia glabra*. Hydrolytic enzymes, pectinases and cellulases play a role in the mechanisms by which endophytic bacteria penetrate into and persist in the host plant (Hallmann *et al.*, 1997; Reinhold-Hurek and Hurek, 1998a). The cell wall of the host plant contains cellulose, whereas the middle lamella between
cell walls contains mainly pectin (Hallmann et al., 1997). In this study 84% of the isolates were able to produce cellulase.

In the present study, pectinase activity did not record very clear zone in any of the isolates. During the growth of the bacterial colony on pectin medium, a clearing was detected just beneath the colonies indicative of the ability of the bacteria to utilize pectin to the extent of requirement. Hung and Annapoorna (2004) also reported cellulase and pectinase activity in the endophytic isolates of soybean. Pectinase activity was reported in the endophytes of tomato by Patel et al. (2012). Pectinases catalyzes the hydrolysis of methyl-ester groups of cell wall pectins. The ability of endophytes to degrade pectate could play a role in colonizing the interspatial region between plant cells (Taghavi et al., 2009).

5.3. Functional properties of the endophytic bacterial isolates

5.3.1. Polysaccharide production

Bacterial colonization relies on a variety of cell surface-associated factors that allow adhesion to the host surface. Exopolysaccharide production play an important role in root adhesion and subsequently plant colonisation. In the present study, 57% of the isolates produced exopolysaccharide. The ragi isolate ECE5 and sunflower isolate HAE8 produced substantial amount of polysaccharide after 24 hours of inoculation. Whang (2007) isolated fifty endophytic bacteria, which produced slime around the colonies, from Pueraria roots. Burkholderia brasiliensis, a diazotrophic endophytic organism isolated from rice roots was reported to produce exopolysaccharides by Mattos et al. (2001). In the present study however the rice isolates did not produce any polysaccharide.
5.3.2. Nitrogen fixation ability

The concept of Biological Nitrogen Fixation by endophytes has been introduced by Cavalcante and Do¨bereiner (1988) and mostly tested with graminaceous plants. They suggested that endophytic bacteria better express their nitrogen fixation potential inside plant tissues due to the lower competition for nutrients and protection against high levels of O2 present on the root surface. In the present study, 73 % of the total isolates were able to grow in N free media. In this study, the soybean isolates GME15 (Bacillus sp.) and GME16 (Pseudomonas sp.) as well as the groundnut isolate AHE3 (Bacillus sp.) could grow in N free media while the cowpea isolates VUE13 and VUE14 did not grow. The ragi isolates ECE4 (Bacillus sp.) and ECE6 (Klebsiella sp.), sunflower isolate HAE8 (Klebsiella sp.), chilli isolates CAE10 and CAE11, and tomato isolate LEE19 (Klebsiella sp.) exhibited good growth in N free media.

The occurrence of Bacillus species as nodule endophytes with N fixing potential has been reported for soybean by Bai et al. (2002). In the present study also three of the isolates which could grow in N free media belonged to Bacillus sp. Asis et al. (2004) determined the population of N2-fixing endophytes in the sugarcane stem and the population density of N2-fixing endophytes ranged from 10³ to 10⁶ cells/ml apoplast solutions. They are reported to improve root growth and function, often leading to increased uptake of water and mineral nutrients (Matiru and Dakora, 2004).

5.3.3. Phosphate solubilization ability

The ability of bacteria to solubilize mineral phosphates has been of interest to agricultural microbiologists, as it can enhance the availability of phosphorus for microbial and plant growth. Bacteria capable of producing a halo/clear zone due to solubilization of inorganic phosphate in the surrounding medium were selected as potential phosphate
solubilizers. In the present study, 78.95% of the isolates were found to solubilise inorganic phosphate to varying degree on Pikovskaya agar and Sperber’s agar. VUE13 identified as *Bacillus megaterium* showed the highest solubilisation of Pi. HAE7 (*Acinetobacter sp.*), HAE8 (*Klebsiella sp.*) and AHE3 (*Bacillus sp.*) were good Pi solubilisers. Phosphate-dissolving endophytic bacteria belonging to *Bacillus, Pseudomonas, Klebsiella* and *Acinetobacter* were isolated from maize and rape plants by Huang *et al.* (2010). Stajković *et al.* (2009) reported the endophyte, *Sinorhizobium meliloti* as a P solubiliser in alfalfa with a zone of 9mm. ThamizhVendan *et al.* (2010) studied the endophytes of Ginseng plants and the endophytic isolates, belonging to *Bacillus cereus* and *B. megaterium* showed notable P solubilization activity. Long *et al.* (2008) reported six endophytic isolates of *Solanum nigrum* that solubilize inorganic phosphate in Pikovskaya agar.

As the plate assay is not considered a reliable method in determining a strain as phosphate solubilizer, the pure cultures were further screened in liquid medium containing Ca$_3$(PO$_4$)$_2$, at a concentration of 5 g L$^{-1}$ as insoluble P source. In the present study, the highest phosphate solubilisation potential was shown by the isolate VUE13 (*B. megaterium*) (6.56 µg). All the isolates obtained except the chilli isolates and the rice isolate OSE2 showed some inorganic phosphate solubilisation. The amount of phosphate solubilized by sugarcane endophyte was observed to be 21 µg P in a report by Gangawar and Kaur (2009). 63 endophytic isolates of maize belonging to *Pseudomonadaceae* were reported to solubilise phosphorus by Johnston-Monje and Raizada (2011). Patel *et al.* (2012) reported phosphate solubilisation ability of the bacterial endophytes of tomato.

In the present study the oilseed isolates AHE3 (*Bacillus sp.*), HAE7 (*Acinetobacter sp.*) and HAE8 (*Klebsiella sp.*) were found to produce
exopolysaccharides and exhibited growth in N free media. They also showed good phosphate solubilization potential. This may be because the presence of exopolysaccharides creates a condition favourable for N fixation. These isolates produced acid in glucose fermentation test which is reflected in the enhanced phosphate solubilization exhibited.

5.4. **Intrinsic antibiotic resistance of endophytic isolates**

The Intrinsic antibiotic resistance (IAR) of endophytic bacterial isolates was studied to find out the variability in the resistance characters of the isolates and further utilize this character for developing an antibiotic marker strain. In the present study, the intrinsic antibiotic resistance was determined against two antibiotics, kanamycin and streptomycin at four different concentrations viz., 50, 100, 200 and 300 ppm. All the isolates were resistant to 50 ppm and 100 ppm of both the antibiotics. The cowpea isolates and chilli isolates were resistant to 150 ppm of both while cowpea isolate VUE13 and the soybean isolate GME16 were resistant to 200 ppm Kanamycin. The isolates from pulses, chilli and cereals had more antibiotic resistance than tomato and oilseed isolates.

Similar results were recorded by Zinniel *et al.* (2002) where 36 % of the endophytic isolates of sorghum, wheat, maize and prairie plants were resistant to streptomycin and 70 % were resistant to Kanamycin. In a study by Gangawar and Kaur (2009) fifteen sugarcane endophytic isolates were sensitive to gentamycin, kanamycin and streptomycin. The IAR is a mechanism in microbes which helps it to tide over stress situations. The IAR is due to the presence of genes which are responsible for the synthesis of enzyme systems, present both in the main chromosome and plasmids that detoxify the antibiotic and proteins that inhibit the cellular transfer of the antibiotic (Hayes and Wolf, 1990).
5.4.1. Development of antibiotic marker strain of endophyte by irradiation

To confirm that the isolates obtained was really endophytes, a marker strain was developed for antibiotic resistance. The antibiotic resistance of GME16 against Kanamycin increased to 500 ppm by UV irradiation and that strain was used as a marker strain for reisolation experiments. Stone, Wyss, and Haas (1947) have reported a marked increase in resistance of staphylococci to penicillin and streptomycin immediately following ultraviolet irradiation. Newcombe and Whitehead (1951) have also reported increased resistance to antibiotics following irradiation of Micrococcus pyogenes var. aureus. Irradiation of E. coli, strain K-12, caused a striking increase in the proportion of cells resistant to intermediate streptomycin concentrations (4 to 10 ug per ml) in an experiment by Saz et al. (1952).

In the present study, when the isolate 200 KV(VUE13) and 200 KG (GME16) which were naturally resistant to 200ppm Kanamycin were exposed to ultraviolet irradiation for 15 minutes the antibiotic resistance of the isolates 200 KV increased to 400 ppm and 200 KG increased to 500 ppm of Kanamycin. The single colonies of 200KG that came up in the 500 ppm Kanamycin plates were picked up and pure culture was maintained in 500 ppm Kanamycin media. These were considered as antibiotic resistant mutants and designated as marker strains for Kanamycin resistance and labeled 500KGX. Antibiotic resistance was assessed as a virulence factor by Lata et al. (2006) and reported that majority of endophytic bacteria were resistant to the antibiotic kanamycin, but susceptible to chloramphenicol. In the present study also two isolates were found to be resistant to Kanamycin.
5.4.2. Establishment of marker strain 500KGX in the seeds and shoots of soybean

In the present study, the colonization and reisolation of 500KGX from its host plant soybean was studied. The initial inoculum population for seed bacterisation was $16 \times 10^7$ cfu/ml. After 7 days 500KGX was resiolated on 500 ppm Kanamycin media from surface sterilized shoots of germinated seedlings and the population was found to be $27 \times 10^3$ cfu/g. Thus, it was confirmed that these isolates were true endophytes as the surface inoculated isolate was reisolated from within the stem tissue. In a similar experiment Burch and Sarathchandra (2006) reported the colonization and recovery of spontaneous rifampicin-tolerant mutants (Rif+) of endophytic bacteria in clover plants. Five Rif+ strains were individually inoculated onto white clover seedlings and all five were reisolated from shoots after 6 weeks and the population of Rif+ bacteria in the shoots was $5 \times 10^2$ cfu/g. Colonization of sorghum and wheat after seed inoculation with *Gluconacetobacter diazotrophicus* strains (containing the marker gene *gus*A) was studied by Luna *et al.* (2010). They observed endophytic colonization over a range of inoculum levels (from $10^2$ to $10^6$ cfu per plant) without significant differences in the extent of colonization indicating that few cells were enough for plant entry and further interior colonization. *G. diazotrophicus* could be reisolated from homogenated surface-sterilized roots and shoots indicating internal colonization and spreading within the plants.

5.5. Role of endophytes in plant growth promotion

5.5.1. Effect of marker strain 500KGX on seedling growth of tomato

Every plant found so far associate with at least one kind of endophytic microbes. Endophytes colonizing inside plant tissues contribute to the fitness of host and in return, they gain nutrient and protection from the host (Rosenbleuth and Martinez Romero, 2006). To
reveal the effects of endophytes, inoculation experiments have been performed, but it has been a problem to eliminate resident or indigenous endophytes from plants in order to have bacteria-free plants or seeds.

In the present study, an investigation was conducted to evaluate the necessity of endophytes for plant growth. This was carried out by using surface sterilized seeds of tomato that eliminated all the epiphytic organisms. The endophytic microorganisms were removed by growing the seeds in antibiotic agar. The results indicated that the surface sterilized seeds grown on antibiotic agar (where all the epiphytic and endophytic microorganisms were eliminated) showed the least vigour index (288). Normal non surface sterilized seeds recorded vigour index of 480. Seed bacterisation with 500 KGX showed improved germination and recorded highest vigour index (950). Thus it can be concluded that endophytes are essential for normal plant growth and seed bacterisation with endophytes enhances the growth.

5.5.2. Evaluation of endophytic bacteria for seedling growth of paddy

In the present study, all the nineteen isolates along with the reference culture *Azotobacter chroococcum* were tested for their ability to enhance plant growth. All the isolates were found to enhance the seedling growth of paddy. 42% of the total number of isolates recorded more than 60% increase in growth compared to uninoculated control.

Similar results were obtained by other workers also. In a study by Rajendran et al. (2007) endophytic bacteria from the roots of coconut palms EPC5 promoted the rice seedling growth in roll towel and pot culture method and were found to increase the vigour index of rice seedlings significantly when compared to untreated control. A *Solanum nigrum* seedling vigor assay to screen the endophytic bacterial isolates for
their PGP ability, was conducted by Long et al. (2008) and 37 of 77 endophytic isolates increased seedling vigor. Hallman et al. (1997) reported that most of the endophytic bacterial strains are capable of promoting plant growth.

Since the plant growth promotion effect of the isolated endophytic bacteria was established by the above preliminary studies we tried to elucidate the mechanisms by which plant growth promotion occurs like production of plant growth regulators and control of phytopathogens.

5.5.3. Production of plant growth regulators by endophytic bacterial isolates

The production of plant growth regulators Gibberellic acid (GA), Indole acetic acid (IAA) and cytokinins (Benzyl adenine) by the endophytic bacterial isolates was studied by bioassay as well as spectrophotometric estimation. In the present study, all the endophytic isolates were found to produce all the three plant growth regulators in the media. The GA concentration in 25 ml of the culture filtrate varied from 9.91 µg in the soybean isolate GME16 to 1.1 µg in sunflower isolate HAE7. The highest IAA production was by the tomato isolate LEE19 (212.30 µg of IAA) while 50 % of the isolates produced ≤ 10 µg/ml IAA. The cytokinin (benzyl adenine) concentration in the culture filtrate was highest in the tomato isolate LEE18 (5.816 µg) followed by soybean isolate GME16 (3.503 µg). Thus the results of the present study indicate all the endophytic isolates obtained could produce GA. The cereal and vegetable isolates were found to produce more IAA while the isolates from pulses and vegetables showed more cytokinin production. The results of the present study are in line with earlier studies.

Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-
defined culture media was reported by Chen et al. (1998). Hung and Annapurna (2004) reported out of 65 endophytes of soybean, 15 produced IAA more than 25 µg/ml. Jha and Kumar (2007) reported seven of 10 endophytic isolates of *Typha australis* were positive for IAA production. Two bacterial endophytes eliciting root plant growth promotion by IAA production and plant defense on pepper (*Capsicum annuum* L.) were isolated by Kang et al. (2007). Long et al. (2008) reported the production of IAA in the range of 1.1 to 154 µg/ml by the endophytic isolates of *Solanum nigrum*. In a report by Gangawar and Kaur (2009) fifteen endophytic isolates from sugarcane produced the phytohormone IAA, which ranged from 4 to 19.3 µg/ml. Thamizh Vendan et al. (2010) studied the endophytes of Ginseng plants and reported that except four all the isolates produced higher amounts of IAA (13.93 µg/ml). Piccoli et al. (2011) reported that the endophytic diazotrophic bacterium isolated from roots of the halophyte shrub *Prosopis strombulifera* produced ABA, IAA, GA1 and GA3 in chemically-defined culture medium as assessed by GC-EIMS.

### 5.6. Compatibility of endophytic bacterial isolates with beneficial soil microorganisms

All the endophytic bacterial isolates were compatible with the beneficial soil microorganisms *Pseudomonas sp.*, *Azotobacter sp.*, *Bacillus subtilis* and *Bacillus megaterium* in the present study. As the isolates belonged to *Bacillus sp.* and *Pseudomonads* predominantly they were compatible with these beneficial organisms. Dual compatibility of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Pseudomonas corrugata* was evaluated by Georgokapoulos (2002) and found to be compatible in broth culture. In a study by Rajendran et al. (2007) *Pseudomonas sp.* showed more compatibility with *T. viride* and *Bacillus sp.*
5.7. Biocontrol efficiency of endophytic isolates against fungal pathogens of vegetables

The widely recognized mechanisms of biocontrol mediated by endophytes are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens and/or abiotic stresses. In the present study, endophytic bacterial isolates were screened for in vitro growth inhibition of four phytopathogenic fungi Colletotrichum sp., Fusarium sp., Rhizoctonia sp., and Pythium sp by dual culture method in plate assay and broth culture. The rice isolate OSE2, and the chilli isolates CAE9 and CAE10 inhibited all the phytopathogens tested. The root pathogens were inhibited by the rice isolate OSE1, ragi isolate ECE4 and ECE6, sunflower isolates HAE7 and HAE8, Chilli isolates CAE11 and CAE12, VUE14 and the tomato isolates LEE17, LEE18 and LEE19. Similar results were reported by other workers from the endophytes of crop plants. Mew and Rosales (1986) reported that 91% of the endophytic isolates of rice inhibited the mycelia growth of Rhizoctonia solani in vitro and the zone of inhibition ranged from 4 to 30 mm. In dual culture assay of 72 endophytic bacteria strains of tomato, 49 strains could inhibit Botrytis cinerea (tomato grey mould disease) in varying degrees (78% in dual culture assay and 100% using fermentation filtrate) according to Yang et al. (2011). Patel et al (2012) recorded antifungal activity of the endophytic isolates of tomato against Fusarium oxysporium, Alternaria sp., Trichoderma sp. and Rhizoctonia solani in plate assay.

In the present study, 50% of the isolates recorded more than 90% inhibition of Colletotrichum sp. Out of the total 19 isolates, eight inhibited Fusarium sp. to 80%, Rhizoctonia sp. to 50% and Pythium sp. to 70%. Rajendran et al. (2007) reported that Bacillus sp. and Pseudomonas Pf1 effectively inhibited the growth of Ganoderma lucidum in vitro. Teng et al.
(2010) reported ACC deaminase-containing endophytic bacteria of *Pseudomonas sp.* and *Pantoea sp.* isolated from halophyte *Suaeda salsa* have abundant biocontrol activity against *Fusarium sp.*

The results of seed bacterisation to control damping off of root pathogens in tomato indicate that the rice isolate OSE2 identified as *Citrobacter sp.*, ground nut isolate AHE3 identified as *Bacillus sp.*, chilli isolates CAE10 (*Citrobacter sp.*) and CAE11 (*Pseudomonas sp.*) and the tomato isolates LEE17 (*Vibrio sp.*) and LEE18 (*Pseudomonas sp.*) could control all the three pathogens. Earlier workers have also reported similar inhibition of pathogens by endophytes.

In a study by Mew and Rosales (1986) rice plants had significantly less disease incidence of *Rhizoctonia sp.* when grown from seeds treated with endophytic bacteria than plants from non treated seeds. Bhowmik *et al.* (2002) reported that seed bacterization with endophyte, Endo PR8 was found to be the most effective to reduce the cotyledonary infection by *Xanthomonas sp.* The endophytic *Bacillus spp.* CY22 isolated from balloon flower produced iturin A with antifungal activity against *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum* (Cho *et al.*, 2003). Chen *et al.* (1995) showed that of 170 bacterial strains isolated from the internal tissues of cotton, 40 possessed biological control activity against *Rhizoctonia solani* in cotton, and 25 induced systemic resistance to *Colletotrichum orbiculare* in cucumber. The intimate relationship between endophytic bacteria and their hosts make them natural candidates for selection as biocontrol agents (Chen *et al.*, 1995) and would obviate the need for selecting bacterial types with high levels of rhizosphere competence often considered necessary for successful seed or root bacterization treatments before or at planting.
5.8. Amplification of nitrogenase gene (*nifH*) by PCR

Since the *nifH* gene only occurs in nitrogen fixing microorganisms, it has been used to monitor the presence of these diazotrophs in pure cultures. Studies employing different *nifH* primers have shown successful and specific amplification of *nifH* from a variety of bacteria and natural samples (Frank *et al.*, 1998).

In this study, the presence *nifH* nitrogenase in the tomato isolate LEE19 was detected by PCR amplification of the expected *nifH* gene fragments (about 360 bp). The *nifH* gene, which codes for component II (Fe protein or nitrogenase reductase) of the nitrogenase enzyme complex, is conserved and can be used to detect the presence of nitrogen-fixing bacteria (Han *et al.*, 2004). In the present study, the isolate LEE19 tentatively identified as *Klebsiella* sp. along with the reference culture *A. chroococcum* amplified the nitrogenase gene (*nifH*) of 360bp. Iniguez *et al.* (2004) reported nitrogen fixation in wheat (*Triticum aestivum* L.) upon inoculation with a nitrogen-fixing bacterium, *Klebsiella pneumoniae* 342 and confirmed the presence of *nifH* in *K pneumoniae* 342 by PCR.

Several groups of endophytic N\textsubscript{2} fixing bacteria have been identified in agronomic crops, such as *Azotobacter*, *Azomonas*, *Beijerinckia*, *Dercia*, *Azospirillum*, *Aquaspirillum*, *Thiobacillus*, *Pseudomonas*, *Xanthobacter*, *Rhizobium*, *Methylosinus*, *Mycobacterium*, *Klebsiella*, *Erwinia*, *Enterobacter*, *Citrobacter*, *Escherichia* and *Bacillus* (Mark and Crasswell, 1992). James and Olivares (1998) reported that endophytic diazotrophs may actually fix N\textsubscript{2} *in planta* and transfer the fixed N products to their hosts. Much evidence exists for significant N\textsubscript{2} fixation by endophytic diazotrophs such as *Gluconacetobacter*, *Azoarcus*, and *Herbaspirillum* (Reinhold-Hurek and Hurek, 1998b).
5.9. Applications of bacterial endophytes

Greater productivity and competitiveness in agriculture are anticipated to come from increased efficiency through the acquisition and management of new biotechnologies and crop production strategies. A renewed interest in the internal colonization of healthy plants by nonrhizobial endophytic bacteria has arisen as their potential for exploitation in agriculture becomes apparent (Kloepper et al., 1992). Any plants that are propagated vegetatively are likely to have an enduring community of bacterial colonists that are transferred in successive progeny generations. Several methods of delivery of endophytic bacteria to crop plants are reported which includes seed treatment (seed biopriming), bacterisation of plant propagation material, soil application and even foliar application.

5.9.1. Application of endophytes in Nursery technology

5.9.1.1. Effect of endophytic isolates on the rooting and establishment of cuttings of the ornamental plant, *Hibiscus rosasinensis*.

In vegetatively propagated plants like ornamental plants, horticultural crops etc endophytic bacteria can be directly delivered into the succulent plant system prior to the planting in the soil. In the present study the effect of endophytic isolates on the rooting and establishment of cuttings of the ornamental plant, *Hibiscus rosasinensis* was studied. Being a vegetatively propagated plant species, *Hibiscus rosasinensis* shoots are amenable for treatment with endophytic bacteria.

In this study four isolates *viz.*, the ragi isolate ECE6, sunflower isolate HAE7, the tomato isolates LEE18 and LEE19 and the consortia of the four were used. The treatment of cuttings with the tomato isolate LEE19 for 12 hours, (*Klebsiella sp.*) showed 75 % sprouting of cuttings.
and the highest shoot and root parameters compared to uninoculated cuttings. The rooting also increased to 100 % in LEE19 treatment which was similar to that observed in IBA treatment. The tomato isolate LEE18 (Pseudomonas sp.) and the ragi isolate ECE6 (Klebsiella sp.) were the next best isolates with 80 % rooting. The root length and root biomass in these treatments were comparable to that of the application of commercial formulation Quicroot. The best treatment for all the parameters was the tomato isolate LEE19. This isolate was found to produce 220µg of IAA per 25 ml of culture filtrate. Hence each cutting was estimated to imbibe 17.5 µg for the plant hormone which may be responsible for the enhanced rooting in these cuttings. So the pre-plant stem treatment of cuttings can be recommended as a strategy for substituting or supplementing the use of chemical plant growth hormones in vegetative propagation of cuttings.

Similar results were obtained in poplar cuttings by Taghavi et al (2009). They reported that the poplar cuttings inoculated with endophytic Enterobacter sp. strain 638 and Bacillus cepacia BU72 showed statistically better growth than the control plants and showed increased root biomass. Aravind et al. (2012) also reported superior sprouting behavior, number of leaves, root biomass and total biomass of the plantlets of black pepper stem cuttings when inoculated with endophytic Pseudomonas aeruginosa and Bacillus megaterium treatments than the untreated stem cuttings.

5.9.1.2. Effect of endophytes on the growth and establishment of Tissue culture banana var. Nanjangudu Rasabale

Typically, aseptic tissue culture explants are grown under conditions of low light, on artificial media, and in small containers. However, the lack of good gas and moisture exchange, the subsequent repression or modulation of some metabolic pathways, and elevated
levels of vitamins, sugars, minerals, and growth regulators often result in plantlets with reduced photosynthetic capacity, malfunctioning stomata, and root systems lacking in root hairs, poor cuticle development, and low wax deposits (Sturz et al., 1997) Subsequent transplantation into the natural environment has often led to losses from severe environmental stress. The introduction of beneficial bacteria can correct and in some cases improve plant performance under stress environments (Nowak et al., 1995).

The addition of beneficial endophytes, presently considered contaminants by tissue culture facilities may be of significant benefit in the clonal multiplication of banana, potatoes, ornamental flowers and medicinal plants (Sturz et al., 1997). The irony is that these explants are natural hosts to a diverse number of species of endophytic bacteria with plant growth-promoting and disease-retarding capabilities all of which have been removed as a consequence of the introduction of commercial micropropagation systems.

Biotization is defined as the metabolic response of in vitro grown plant material to a microbial inoculant(s), which promotes developmental and physiological changes that enhance biotic and abiotic stress resistance in subsequent plant progeny (Sturz et al., 2000). The incorporation of microbial inoculants into soilless mixes is one potential method of delivering endophytes to plants at an early stage in their growth (Whipps, 2001). Another method is the inoculation of nodal explants in the multiplication steps preceding transplantation to the greenhouse or field (Nowak and Shulaev, 2003).

Hence, the effect of endophytic bacterial isolates on growth and establishment of tissue culture banana was investigated. In the present study, the endophytic bacteria were inoculated to the tissue culture banana at two stages, at the primary hardening stage and at the
secondary hardening stage. The micropropagated plantlets at the time of transfer from bottles to cups (1^0 hardening) and from cups to polybags (2^0 hardening) were inoculated with three endophytic bacterial isolates. These isolates soybean isolate GME16 (*Pseudomonas sp*), tomato isolates LEE18 (*Pseudomonas sp*) and LEE19 (*Klebsiella sp*) were inoculated individually and in consortia. These isolates were selected as they possessed the highest plant growth promoting potential. GME16 (*Pseudomonas sp*) produced the maximum Gibberellic acid, LEE18 (*Pseudomonas sp*) produced cytokinins and solubilised phosphate and LEE19 (*Klebsiella sp*) produced IAA as well as fixed nitrogen.

The results obtained after both stages indicated that biotization enhances the growth parameters of tissue culture plantlets. The parameters like root length, shoot length, no of leaves, leaf area index and the plant biomass significantly increased due to the treatment with the tomato isolate LEE18 identified as *Pseudomonas sp*. The root biomass increased 250 to 400 % due to the treatment with endophytic isolates. Auxins and cytokinins are the two most cited growth promoters associated with the induced growth of plants as a response to endophytic infection. Auxins stimulate cell division, thus explaining the increase in root mass and accelerated formation of root hairs, while cytokinins induce root elongation, thereby also increasing root mass. Cytokinins are also reportedly responsible for enhancing nutrient accumulation and transportation, contributing to overall improved plant growth. The endophyte LEE18 used in the present study was found to be producing cytokinins in significant amounts *in vitro*. The other isolates also produced significantly higher growth hormones.

The growth promoting characteristics of *Pseudomonads* is already established by several workers. In a study by Harish *et al.* (2008) endophytic *Pseudomonas sp* and *Bacillus sp* recorded a significant
improvement in morphological characters in the biohardened plantlets treated with different combination of the endophytic bacterial strains than the control under field conditions. Ting et al. (2008) also reported enhanced growth of tissue culture banana due to endophytic inoculation. Kavino et al. (2007) recorded showed improved vegetative growth, physiological attributes, PR—proteins and phenol contents besides reducing banana bunchy top disease (BBTD) incidence in the field in micropropogated banana. The increase in morphological characters along with other physiological changes are important in the production of micropropagated plantlets since they could reduce the time for acclimatisation and render plants less susceptible to pathogens. Hence from this study the application of endophytes is strongly recommended at the nursery stage on tissue-cultured banana in order to allow the establishment of endophytes prior to transplanting to the field.

Bacterized plantlets not only grow faster, but are sturdier, with a better developed root mass and are significantly more capable of withstanding low-level disease pressure than non-bacterized plantlets (Sharma and Nowak, 1998). The overall effect of a more vigorous plant is increased drought resistance and reduced transplanting shock through improved water management (Nowak et al., 1995). In plant-bacterial coculture, plant growth promotion effects reported include increase in plant height (Chanway et al., 1997), root and shoot biomass (Pillay and Nowak, 1997), lignification of xylem vessels (Frommel et al., 1991), root and leaf-hair formation (Frommel et al., 1991, Nowak, 1995) etc.
5.9.2. Application of endophytes in field

5.9.2.1. Comparison of the effect of plant growth promoting endophytic bacterial isolates on the seedling growth of different varieties of aerobic rice.

The effect of endophytes on seedling vigour of different varieties of paddy, MAS946, MAS 145, MAS 99 and MAS 26 was compared and the germination percentage was 100 % due to seed bacterisation with endophytic isolates than uninoculated control 70 % in all the varieties tested. The vigour index of aerobic rice increased substantially due to endophyte inoculation and the soybean isolate GME16 was found to be the best isolate in the case of all varieties with values ranging from 910 in MAS145 to 1530 in MAS26. GME16 identified as *Pseudomonas sp.* produced more gibberelllic acid (GA) than other isolates. GA stimulates the cells of germinating seeds to produce mRNA molecules that code for hydrolytic enzymes and acts as stimulation for seed germination. Presoaking seeds in GA-3 solution was found to cause the rapid germination of many types of highly dormant seeds which would otherwise need cold treatment. Reports of similar nature on the production of IAA and GA by endophytes have been made by various workers. Vikram *et al.* (2007) reported GA production by *Pseudomonas sp.* Khalid *et al.* (2004) also reported similar results with rhizobacteria. Rajendran *et al.* (2007) reported the endophytic bacterial isolates of coconut and *P. fluorescens* strain Pf1 were found to increase the vigour index of rice seedlings significantly when compared to untreated control.

5.9.2.2. Pot culture studies to evaluate the plant growth promotion potential of endophytic isolates on crop plants

The utilization of endophytic and epiphytic bacteria in agriculture production depends on the knowledge of the bacteria-plant interaction and the ability to maintain, manipulate and modify beneficial bacterial
population under field conditions (Hallmann et al., 1997). Growth promotion by endophytic bacteria seems to be governed by the interactions among internal and external microfloral populations. The order in which endophytic populations (single and mixed) are inoculated and become established in the host plant will affect subsequent plant growth responses (Sturz et al., 2000).

In the present study, three endophytic isolates, GME16 (*Pseudomonas sp*), LEE18 (*Pseudomonas sp*) and LEE19 (*Klebsiella sp*) were inoculated individually and in consortia to three crops tomato, paddy and cowpea as seed treatment and as foliar spray at flowering stage. The inoculation was done along with 100 %, 75 % and 50 % of recommended dose of NPK fertilizers for the individual crop. These isolates were selected for the pot culture studies as GME16 (*Pseudomonas sp*) produced the maximum Gibberellic acid, LEE18 (*Pseudomonas sp*) produced cytokinins and solubilised phosphate and LEE19 (*Klebsiella sp*) produced IAA.

### 5.9.2.2.1. Effect of endophytic bacterial isolates on growth and yield of tomato var. Vaibhav

In the present study, the germination percentage increased due to seed bacterisation with endophyte to 100 %. The treatment with full dose of NPK and the tomato isolate LEE18 was the best for all the seedling parameters studied and the vigour index was 1560 as compared to uninoculated control (632). The plant height also recorded significant increase due to the endophytic treatment and the treatment with full dose of NPK and the tomato isolate LEE18 as well as 75 % NPK and LEE18 recorded 77 % increase over uninoculated control. Even the treatment with the consortia of endophytes alone showed 65 % increase over uninoculated control. The number of leaves and branches also showed a similar trend.
The yield per plant showed significant increase due to the inoculation with endophytes. The treatment with the tomato isolate LEE18 along with full dose of fertilizer showed the highest yield of 705.18 g. With 75 % NPK dose and the LEE18 isolate showed significantly higher yield of 650 g. Thus due to the treatment with LEE18 and full NPK there was 15.76 % increase in yield with respect to NPK control and 173.3 % increase with respect to absolute control. 30.8 % increase in yield was recorded due to the treatment with consortia of endophytes alone compared to absolute control. The dry matter production showed a similar trend. In the treatment with the tomato isolate LEE18 along with full dose of NPK the dry matter production increased 6.63% over NPK control and 47.63% over absolute control. LEE18 with 75% NPK showed 2% increase over NPK control and 41.12% over absolute control. The treatment with consortia alone was better than absolute control by 7.5%. Thus 25% fertilizers can be replaced if seed bacterisation with endophytes is followed as a cheap alternative to improve yield.

In the present study, the tomato isolate LEE18 identified as *Pseudomonas sp.* was responsible for the plant growth promotion and yield increase. Bhatia *et al.* (2008) reported the fluorescent pseudomonads when used as inoculants in groundnut, enhanced germination up to 15 % and 30 % with subsequent increase in grain yield upto 77 %. Algam *et al.* (2005) reported all the endophytic isolates of tomato when applied as seed treatment + soil drench enhanced the height of the plant, fresh weight, dry weight and number of fruits when compared to the non-treated control. From the results it is evident that bacterial endophytes introduced as seed treatment colonize the internal tissues of root radicles as they emerge from the seed coat, as previously reported by Rajendran *et al.* (2007). Foliar spray treatment was effective to inoculate bacterial endophytes in the leaf tissues (Algam *et al.*, 2005).
5.9.2.2.2. Effect of endophytic bacterial isolates on growth and yield of paddy var. MAS 99.

In the present study with paddy var. MAS99, all treatments with endophytic bacterial isolates recorded 100 % germination while the absolute control, NPK control, and the treatments with consortia recorded 90 % germination. Seedling vigour recorded significant difference among the treatments and the highest was recorded in the treatment with the soybean isolate GME16 (*Pseudomonas sp.*) and full dose of NPK (1933). In a study by Chandrasekhara (2007), all the endophytic bacterial isolates significantly enhanced the seed germination and seedling vigor of pearl millet and the highest germination percentage and vigor index was recorded for the *Pseudomonas* isolates.

At all intervals, the highest plant height was recorded in the treatments with full dose of NPK. The soybean isolate GME16 inoculation showed highest plant height (90.67 cm) followed by the tomato isolates LEE18 (89.33 cm) and LEE19 (88 cm). Similar trend was recorded in the number of leaves and number of tillers.

The yield per plant recorded significant difference among treatments. The best treatment for improving the yield of paddy was found to be the treatment with soybean isolate GME16 (*Pseudomonas sp.*) with full dose of NPK which recorded 19.56 g. The treatment with consortia alone was on par with the treatment with 50 % NPK (7g). Absolute control recorded the least yield of 5.48 g. Thus it can be concluded that the yield of paddy var. MAS99 significantly increased due to the treatment with endophytes. The isolate GME16 was found to be the best when applied with full dose of NPK which recorded 95.6 % increase over NPK control and 257 % over absolute control. LEE18 (*Pseudomonas sp.*) with full dose of NPK showed 58.3 % increase over NPK control and 188 % over absolute control while LEE19 (*Klebsiella sp.*)
with full dose of NPK recorded 22.5 % increase in yield over NPK control and 123.5 % increase over absolute control. With 75 % NPK and GME16, the yield increase was 23.2 % which was on par with that of full dose of NPK and LEE19. Also 75 % NPK with LEE18 as well as with LEE19 recorded on par yield with that of NPK control. The dry matter production also showed a similar trend. The treatment with GME16 and full dose of NPK significantly increased the dry matter production and recorded 144 % increase over absolute control and 9 % over NPK control. Full NPK with LEE18 and 75 % NPK with GME16 recorded the same dry matter of 58.45 g and recorded 6% increase over NPK control. This points to the fact that these endophytes can substitute for 25% NPK.

Earlier workers have also reported plant growth promotion by endophytes in rice. In a study by Chandrasekhara et al. (2007) in pearl millet all the tested endophytic bacterial isolates were significant in promoting the vegetative growth parameters such as height of the plant, fresh weight, dry weight and number of basal tillers over control due to seed treatment. The maximum improvement of all reproductive traits was noticed in plants raised from seeds treated with the endophytic Pseudomonas fluorescens.

When inoculated, strains of the endophyte, Stenotrophomonas enhanced plant biomass production in corn, sorghum, canola, potato and poplar, all cultivated under greenhouse conditions (Hardoim et al., 2012). In a study by Govindarajan et al. (2008) the endophytic isolate from rice MGK3 identified as Burkholderia vietnamensis was used to inoculate rice seedlings in comparison with four other diazotrophs. MGK3 alone, and combined with other diazotrophs, performed best under both pot and field conditions. Combined inoculation produced yield increases between 9.5 and 23.6 %, while MGK3 alone increased yield by 5.6 to 12.16 % over the uninoculated control treatment.
5.9.2.2.3. Effect of endophytic isolates on growth and yield of cowpea var. KBC 2

In the present study, the vigour index recorded significant difference among the treatments and highest was in the treatment with soybean isolate GME16 and full dose of fertilizers (4267). The highest plant height was recorded at all intervals in the same treatment followed by LEE18 and full dose of fertilizers. Similar trend was recorded in no of leaves and no of branches. 72 % increase in dry matter production was recorded due to the treatment with GME16 with full dose of NPK over absolute control. 64 % increase was there when treated with 75 % NPK and GME16. There are a few reports on the increased plant biomass of different crop species such as oilseed rape, tomato, maize, sorghum, wheat and rice when endophytic bacteria used as seed and seedling treatments (Nejad and Johnson, 2000; Gutierrez-Zamora and Martinez-Romero, 2001).

The yield parameters like number of flowering clusters/plant, number of fruits/plant, fruit length, pod weight, number of seeds/pod and yield/plant recorded significant difference among the treatments. The best yield was recorded in the treatment with soybean isolate GME16 and full dose of fertilizers recording 83.4 g per plant followed by LEE18 with full dose of fertilizers (67.32 g). 75 % NPK and GME16 recorded a yield of 65.25 g while 75 % NPK and LEE18 recorded 53.86 g which was on par with full dose of NPK and LEE19. Thus it can be concluded that the best treatment with GME16 and full NPK increased the yield by 73.2 % over NPK control and 301 % over absolute control. 35.4 % increase in yield over NPK control was recorded in the treatment with 75 % NPK and GME16. Other treatments with 75 % NPK recorded 2 % increase over NPK control. As the treatments with endophytes and 75 % NPK is giving on par yield with NPK it can be suggested to replace 25 % NPK with bioinoculants in cowpea.
The increased vegetative and reproductive growth recorded in all the three crops due to the treatment with endophytic isolates can be attributed to microbial processes leading to nutrient solubilization of phosphorus, disease resistance and production of plant growth hormones such as auxins, cytokinins and gibberellins (Sturz et al., 1997).

In organic agriculture where we want to avoid the use of chemicals, the seed bacterisation with endophytic bacteria is a cheap and viable option to increase yield. *Pseudomonas sp.* and *Klebsiella sp.* isolated as endophytic bacteria possessed at least one of the different characteristics involved in plant growth promotion. In summary, due to their metabolic versatility, seed-borne bacterial endophytes might increase the fitness of plants, giving the host a competitive advantage over other (indigenous) plant communities and thus might affect whole-ecosystem function. Thus it can be concluded that plant endophytic bacteria contribute to plant growth, disease resistance, and crop productivity.

### 5.10. Conclusions and Future line of work

The endophytic bacteria were present in all the groups of crops studied and more number of endophytes was present in the roots than the shoots. The endophytic microorganisms are essential for all plants for growth promotion. In this study, the potential of endophytic isolates for plant growth promotion was determined by assessing the factors such as phosphorus solubilization, IAA production, Gibberellic acid production, and cytokinin production. Although isolates exhibiting all the plant growth-promoting features simultaneously were rare, the ragi isolate ECE6, sunflower isolates HAE7 and HAE8, chilli isolate CAE11, cowpea isolate VUE13, soybean isolate GME16 and the tomato isolates LEE18 and LEE19 were positive for most of these characteristics. It is possible to supplement the plant endophytic populations by: inoculating
(bacterizing/ biotizing) microplantlets, seed, or vegetative seed pieces prior to planting. Using communities of endophytes with various plant-promoting qualities encourage a more robust crop, with better seedling vigor, plant health, general disease resistance, and the ability to cope with a range of environmental stresses (drought, cold, and pests).

The recent areas where these plant growth promoting endophytes can be used are in the developing areas of forest regeneration, biofuel crops and phytoremediation of contaminated soils. To utilize these bacteria more effectively will require research that involve (1) refining procedures for isolating and selecting bacteria; (2) testing of effective carriers (cultivar specificity); (3) examining modes of entry of endophytic bacteria into the host plant or through the incorporation of a beneficial genetic moiety conferring a certain beneficial trait; (4) determining the mechanisms by which growth promotion, growth inhibition, and induction of disease resistance operate; (5) enhancing host receptivity to endophyte colonization, through germplasm selection; and (6) optimizing complementary crop sequences and their management to enhance the build-up or carryover effect of beneficial endophyte populations (Sturz et al., 2000). The goal should be to select and improve endophytic bacterial populations in crops and their soil sources, and to stabilize them at optimum levels.