SUMMARY OF THE THESIS

One of the main problems of plant growth promoting rhizobacteria (PGPR) as inoculants in practice is that the applied microorganisms often fail to survive, or do not execute their specific function in soil environment. The positive effect of a PGPR strain to one soil plant/ environment does not guarantee its success in another soil sample or host plant genotype. Similar problems were reported for hydroponic and soilless systems of plant growth. Understanding the sources of variability and manipulation of the key factors to achieve high and consistent benefits of microbial inoculation is important to overcome this obstacle. Because a primary mechanism of disease suppression available to fluorescent pseudomonads is antibiosis, it is thought that variable performance might result from variation in production of antimicrobial compounds. Much information about the mechanism and factors affecting the action of antibiotics is available, but a more insight is needed about the ecological interactions taking place in the soil and root environments, which might influence production of the antifungal metabolites. It is also necessary to study the extent of strain to strain variation in the factors influencing antimetabolite production to understand whether the principles understood from model strains could be extrapolated to strains from different sources. This could help to customize the biocontrol PGPR strains for use in particular environments and to understand how to prepare the inoculum formulations for their optimal performance; or the environment can be modified to be more favorable to strains; or strains could be constructed that are independent of environmental signals. With the above in mind the present work involved the following.

Isolation, screening and identification of fluorescent pseudomonad strains:

By using three cycles of plant inoculation studies 83 fluorescent bacterial isolates were obtained from various plant rhizospheres, which were screened based on fluorescence of the colonies. Of these, isolates with moderate-to-good antifungal activity were screened by antifungal assay against the fungus *Rhizoctonia bataticola.* Maximum numbers of strains having higher antifungal activity have been obtained from groundnut rhizosphere followed by cotton and sugarcane rhizospheres. Isolates G19, G20 and G25 have shown antifungal activity much better than the model biocontrol strain *Pf* CHA0. Fluorescent strains were identified as pseudomonads on the basis of specific biochemical tests and molecular

identification based on specific PCR amplification of ITS region. Out of 60 biochemically positive isolates about 40 isolates showed ITS positive. Shannon index of diversity was derived from the relative frequencies of the fluorescent pseudomonads in each sample (which indicates the population density of fluorescent pseudomonads) and it was found that the diversity was high in pigeon pea (0.5) and tobacco rhizospheres (0.269), however highest number of fluorescent pseudomonad isolates were obtained from tobacco, rice, sugarcane and groundnut rhizospheres.

PGPR traits in fluorescent pseudomonad isolates:

IAA production by isolates was between 10 – 22 µg/ml. G1, G3, C7, C2, P1, are isolates from ground nut, cotton, pigeon pea rhizospheres, which showed better production than *Pf* CHA0. With tryptophan supplementation, IAA production ranges between 33-43µg/ml. G25 and G18 strains exhibits high IAA production in presence of tryptophan compared to standard strain Pf-5. Lytic enzymes (as biocontrol traits) in isolates that were studied were protease, chitinase and cellulose. None of the isolates other than the standard strain *Pf*CHA0 showed high proteolytic activity. Isolates like G35 and G36 have showed moderate growth on MR media after incubation period of 7 d, indicating weak chitinase activity, while rest of the isolates are chitinase negative. All the isolates were found to be negative for the cellulase activity. Thus, although the isolates showed very good antifungal activity against the pathogenic fungus *R. bataticola,* majority of them were negative for the production of lytic enzymes which are known for their antagonistic effect against many pathogenic fungi. Antifungal metabolite mediated biocontrol in the isolates was an important PGPR trait studied in this work.

Ethylene reduction ability in isolates was identified by studying the presence of ACC catabolism pathway. Many isolates such as G35, G19, P33, P36, P35 exhibited dense growth on plate containing ACC. Isolate G29 strain showed the highest ACC deaminase activity while standard strain Pf-5 exhibited low ACC deaminase activity. Several isolates such as Pf5, G45, G2, H4 were giving yellow precipitates when 2, 4-dinitrophenyl hydrazine (2, 4-D) was added indicating functional KMBA pathway , which is the intermediate product in ethylene biosynthesis via L- methionine. L- Met is converted to C_2H_4 by the transaminase pathway. Strains H9, G29, G13, G35, and H4 utilized both ACC and KMBA pathway.

During studies on HCN production, isolates like G19, G20 and G35 have shown less intense brown colour while others such as G2, G8, C7, etc. have shown brown colour equally intense as that of *Pf* CHA0. Rest of the isolates did not show change of colour and thus are considered as HCN negative strains.

The study of PGPR traits in the isolates obtained in this work showed strain to strain variation in the presence /absence of the trait as well as in the intensity or extent of the trait as moderate or high. Further more, a comparison with the standard well-characterized strain *Pf*CHA0 showed some strains to be more efficient in certain PGPR characteristics than the well-reported strain. The diversity of these strains with respect to the PGPR traits indicates that better and more efficient strains can be isolated from local soils and put to application in the same niche.

Genotypic diversity in isolates was studied with respect to the variation in the ITS region. All genotypes showed a single PCR-amplified ITS product (580 bp).When the ITS-PCR products were digested with *Hae*III and *Msp*I, *Hae* III was found to be effective cutter and gave distinct bands. The cluster analysis has revealed 13 ITS-RFLP types. All the fluorescent *Pseudomonas* genotypes clustered with the three *Pseudomonas* reference strains. There were two major diversifications in the dendogram: one group representing the standard strain *Pf* CHA0 and its closely affiliated strains such as G36, G29 and P1 while other group is represented by standard strains Pf-5 and Q287 and their closely affiliated strains such as G5, G30.

Detection of antibiotic biosynthesis genes in the fluorescent pseudomonad isolates:

The collection of fluorescent *Pseudomonas* spp., which have shown biological control activity against *Rhizoctonia bataticola in vitro* and found to produce secondary metabolites such as siderophore and HCN, were checked for the presence of representative genes in the pathways for these metabolites. Out of total 27 ITS positive fluorescent *Pseudomonas* strains, 18 were positive amplification for *plt*C, 15 for *phl*D,10 for *hcn*BC and 5 for *prnC*. Strains Q287, Pf-5 ad *Pf* CHA0 were used as controls. PCR based detection worked well to amplify *phlD* in a majority of pseudomonads (including strain Q2-87, Pf-5 and *Pf* CHA0), but performed poorly with some strains e.g. strain G25 and P1, whereas no PCR product was obtained from some isolates *viz.* G29. A single product of about 586 bp in length was

obtained for 9 HCN+ strains. The predicted 438-bp fragment was obtained from DNA of model *P. fluorescens* strain Pf-5, *Pf*-CHA0 and Q287. Predicted band was also obtained in 15 fluorescent *Pseudomona*s isolates. *prnC* gene from fluorescent *Pseudomonas* strains was amplified in five isolated strains. Resemblance of the strains based on the presence of antibiotic biosynthesis genes was found for isolates G45, G46 and G14, which were found to be similar to standard strain *Pf* CHA0 in having the all antibiotic synthesis genes. Similarly strains H4, H9, G5 and G22 were found to be similar to other standard strains Q287 and Pf-5.

Biosynthesis and regulation of antifungal metabolites in fluorescent pseudomonad isolates:

2,4 – DAPG biosynthesis and regulation in the isolates : 2, 4- DAPG production by rhizospheric isolates was analyzed using HPLC and was compared to that of model strain *Pf* CHA0. Certain isolates like G1, G2, G8, G3 and C2 showed 2, 4- DAPG production better than *Pf* CHA0 and correlated well with bioassay with *S. aureus* and *R. bataticola*.2, 4- DAPG production of various isolates was checked in the presence of different C sources and P levels. In presence of sucrose, isolates G8 and G1 showed better 2,4- DAPG production than *Pf* CHA0. Isolate G2 showed the less growth is in presence of mannitol, but the 2, 4- DAPG production in presence of mannitol is higher than that in sucrose where growth is good. Glucose enhanced 2, 4- DAPG production in *P. fluorescens* Pf-5, CHA0 but not glycerol and sucrose and except in only *Pf* F113 production of 2,4- DAPG is stimulated by sucrose. In case of *Pf* CHA0 DAPG levels were decreased at 10 mM inorganic phosphate level. The 2,4- DAPG production by G2 was very similar to *Pf* CHA0 showing high production at low Pi concentrations, however isolates G1, G2, and G8 showed higher 2,4- DAPG production than *Pf* CHA0 in absence of any supplemented phosphate. Isolates G8 and G1 showed comparatively good 2,4- DAPG production even at high Pi levels. Isolate G2 showed minimum inhibitory effect of Pi on 2,4- DAPG production and has shown good production up to 50 mM Pi. These results point out the variation in the regulation of antibiotic synthesis in different strains and imply that this may result in differences in their performance in plant rhizospheres where the availability of C and P will depend on plant and soil type.

PRN and PHZ Biosynthesis in isolates: Pyrrolnitrin production was found to be highest in G35 and followed by G25 and G26. Primary screening of phenazine production was done in pigment production medium (PPM) and strains like G16, G 44, G36, G45, H4 showed a dark orange pigment that is characteristic of 2-OHPCA. Other strains like P4 developed a green pigment after 24 h. Preliminary screen for extracellular redox molecules was accomplished using a layered MnO_2-based plate assay, strain's ability to convert insoluble Mn(IV) to colorless Mn(II). Some strains showed high amount of clearance on MnO_2 plates indicating they reduce brown coloured insoluble Mn(IV) to colorless Mn(II) . G44 showed the highest amount of clearance, while green pigmented P4 also showed high clearance. Isolates were characterized as PCA producers (Q287, C7 , G 5, G36, G38, G45, H4, P33) and PCN producers (G 14, G 16, G 44, P1, P4). Based on primary and secondary screening, strain G44 was considered as a high PCN producer.

Effect of nutritional factors on PCN production: Effect of nutritional factors on the production of phenazine-1-carboxamide (PCN) in G44.

Effect of nitrogen sources on PCN production by G44: Effect of nitrogen sources on PCN production were studied after replacement of N source in MVB1-glucose-cas by other sources. Fungal inhibition was used to analyze amount of PCN produced. 8mM NH_4Cl showed maximum antifungal activity. Increase in NH_4Cl levels to 16mM and 32mM showed a marked decrease in PCN production.$(NH_4)_2SO_4$ did not significantly contribute to PCN production at any concentration although an increase in concentration of $(NH_4)_2SO_4$ from 4mM to 8mM decreased PCN production by 33% but a further increase in concentration to 16mM did not show any change. Supplementing the original nitrogen source with urea or $NaNO_3$ did not show increased PCN production. The original medium contained 0.05% casamino acids and an increase in casamino acids concentration to 16mM lead to marked increase in PCN production.

Effect of amino acids on PCN production by G44: To test whether stimulatory effect of casamino acids on PCN levels can be ascribed to individual amino acids, casamino acids were replaced by individual amino acids. Aromatic amino acids, phenylalanine and tryptophan were supplemented as nitrogen source in order to check if they enhanced PCN

production. However, results of antifungal activity of PCN extracts did not show marked increase compared to other N sources.

Effect of carbon sources on PCN production by G44: To study the effect of various carbon sources on the level of PCN production, G44 was grown in MVB1-cas supplemented individually with C-equivalent concentrations of different carbon sources. MVB1-cas supplemented with glycerol showed maximum fungal inhibition. Highest PCN production/ antifungal effect of casamino acid as nitrogen sources while fructose, sucrose and glucose showed moderate inhibition. Supplementing citric acid and malic acid as carbon sources in the original medium showed very little fungal inhibition and therefore minimum PCN production. Similar to our strain, the production of PCA by *P. fluorescens* 2-79 and PCN production by *P. aeruginosa* were found to be stimulated by glucose and glycerol. This suggests a similar response of phenazine-producing pseudomonads to these carbon sources.

Effect of combinations of nutritional factors on antifungal metabolite production in fluorescent pseudomonads by statistical analysis:

Understanding the factors that regulate the biosynthesis of antimicrobial compounds by disease suppressive strains of *P. fluorescens* is an essential step towards improving the level and reliability of their bio-control activity. Quantitative and/or qualitative differences in the sugar, nitrogen, phosphate and mineral components of root exudates could determine the effectiveness of bio-control in given crop-pathogen systems. The effect of seven nutritional factors (glucose, citrate, KNO_3, KH_2PO_4, $FeSO_4$, $ZnSO_4$ and $(NH_4)_6Mo_7O_{24}$ on the growth, antifungal activity and production of 2,4- DAPG, PLT and PRN, by model biocontrol strain *Pf CHA0* was studied using a novel approach of statistical design. Effect of each factor and their combinations on overall growth and antifungal activity as well as individually 2, 4-DAPG, PRN, PLT and PHZ production was studied by regression analysis using DE 8.

***Effect of nutrient combinations on growth and antifungal activity of Pf CHA0*:** Effect of factors on antifungal activity showed many variations in effect (+/-) and their contribution on growth. Possible reason could be that the growth is result of basic metabolism and cumulative effect of many supportive pathways but antifungal activity is contributed by limited number of pathways so the variation in effect was observed. Although C sources

differentially influence medium acidification during growth, which may then indirectly affect antibiotic biosynthesis and bio control activity, but in our result we did not observe any significant change medium pH after day 2 and day 5 of growth. The medium amended with a combination with Zn, Mo and nitrate did not support growth possibly because of very low C/N ratio (C/N<1). Combinations amended with citrate, $FeSO_4$, glucose and KH_2PO_4 and combination amended with glucose, KH_2PO_4, KNO_3 and $ZnSO_4$ have supported high growth on both the days of sampling with OD $_{600nm}$ value of 4±0.5. Combinations amended with citrate, $FeSO_4$, glucose and KH_2PO_4, with citrate, $FeSO_4$, KNO_3 and $ZnSO_4$ and the combination amended with citrate, glucose, $FeSO_4$, KH_2PO_4, KNO_3, $(NH_4)_6Mo_7O_{24}$ and ZnSO4 have shown highest antifungal activity of 70% on day 5 and high growth (OD $_{600nm}$ = 3±0.5). Combination amended only with all inorganic components viz., $FeSO_4$, KH_2PO_4, $(NH_4)_6Mo_7O_2$, and $ZnSO_4$ showed green fluorescent pigment biosynthesis and antifungal activity up to 40% on day 5. Glucose and citrate individually and in combinations, supported well to growth and antifungal activity. Contribution order of factors acting positively to antifungal activity on day 2 is, glucose>citrate>citrate +glucose while on day 5 antifungal activity the order is, citrate> citrate +glucose>Fe^{2+}>glucose>citrate +Pi=citrate+Fe^{2+}. Combination with Fc^{2+} and citratc has shown incrcascd antifungal activity on day 5 and was thought to be mediated by induction of antifungal traits specifically. It is in support of previous observation that iron stimulates bio-control activity and cyanide production. Citrate and Pi individually have positive effect on the growth but their combination have shown negative effect which show a kind of the shift of bacterial physiology/behavior to nutrients present individually and in combinations. Combination without any amendments (considered as negative control) showed low growth, but higher antifungal activity on day 5 than on day 2, which suggest that in diluted nutrient broth antifungal trait gets induced on day 5. Citrate, glucose, Zn^{2+} individually, citrate along with glucose and triple combination of citrate, glucose and nitrate have shown positive effect on growth. Citrate and Pi, citrate and zinc, Mo^{2+}, citrate and Mo^{2+}, triple combination of citrate, glucose and KNO_3 have shown negative effects on growth. In our result, citrate and glucose individually and in combination, have shown strong positive effect on *Pf* CHA0 growth on day 2 and day 5, which is in accordance to previous finding that an organic acid or a tricarboxylic acid cycle intermediate, not glucose, is usually the preferred carbon source in *Pseudomonas* spp. Of the minerals, Zinc

was found to be supportive much to *Pf* CHA0 growth on day 2 and day 5. Ammonium molybdate has been reported to be a strong inhibitor of acid phosphatase activity and the process of phosphorylation/dephosphorylation plays a crucial role in many metabolic processes.

Likewise different combinations have been found to affect different antibiotics. Principal component analysis (PCA) analysis for the five variables with sixteen different combinations was done. Based on, Pearson correlation and Eigen values were obtained which shows the degree to which the variables are related with each other. For the 2d of sampling, the biplot graph shows that on this day of sampling antifungal, OD_{600nm} and PLT biosynthesis showed strong correlation while DAPG and PRN fall in other zone (+x, +y). Day 2 antifungal is positively correlated with PLT maximally (n=0.878) followed by OD_{600nm} (n=0.544), PRN and DAPG. Day 2 PRN has shown positive correlation with OD_{600nm} (n=0.44), antifungal activity (n=0.38). DAPG is nearly independent of OD_{600nm} and PLT (n<0.1). Day 2 PLT has shown high positive correlation with OD_{600nm} (n=0.694) and antifungal activity. Day 5 antifungal activity has shown positive correlation with OD_{600nm} (n=0.522), PRN (n=0.768) and PLT (n=0.475). Day 5 PLT has shown positive correlation with OD (n=0.33) and antifungal activity (n=0.475) but negative correlation with 2,4- DAPG (n= -0.16).2,4- DAPG has shown negative correlation with OD_{600nm} (n= -0.187), PRN (n=-0.131) and PLT.

For day 2, the results showed that three components together accounted for about 90.71% of the total variance and the rest of the components only accounted for about 9.29% first three components, the first component accounted for about 54.52%, the second component about 22.36% and the third component about 13.88% of the total variance in the data set.

For day 5 biplot graph shows that PLT and OD_{600nm} shows correlation and falls in same zone (+x, +y), antifungal and PRN falls in other zone (+x,-y). For the day 5, PCA analysis for first three components, these three components together accounted for about 87.89 % of the total variance and the rest of the components only accounted for about 12.11%. The first component accounted for about 45.72%, the second component about 22.72% and the third component about 19.44% of the total variance in the data set.

Effect of nutritional factors on the antifungal activity by G20: Strain G20 was selected for performing the experiment same as that with *Pf* CHA0 as G20 showed better antifungal activity than *Pf*CHA0 and 16 nutrient combinations experiment was carried out with it. Isolate G20 has shown higher antifungal activity on 2nd day than that of 5th day by ethyl acetate extract. Nutrient combinations which have worked with well with *Pf* CHA0 viz. nutrient combinations number 5, 6 and 8 were studied and it was found that nutrient combinations number 5 and 8 did not support the antifungal activity of strains whereas nutrient combinations 6 has supported to the antifungal activity. This indicated an important finding about the strain to strain differences in the nutritional requirements for optimal antifungal activity. Nutrient combination number 6 has shown percentage inhibition above 50%. Iron and citrate are two factors common in all these flasks so it could be concluded that these factors probably have positive effect on the biocontrol physiology of the isolate G20.

Effect of antifungal metabolites on rhizobia:

Rhizobial strain ST1 was found to be sensitive to DAPG, PRN and PLT, *R. leguminosarum* was inhibited by DAPG and PHZ. Strain IC 3169 is inhibited by DAPG and lesser extent with PHZ, IC 3123 did not get inhibited by 2, 4- DAPG, PRN, PLT. The results show that most of rhizobial strains get inhibited by pure 2, 4- DAPG, PRN, PLT. Dual culture tests for rhizobia –fluorescent pseudomonad interaction showed that rhizobial strains IC3169 and *R. leguminosarum* 3841, *S. meliloti* were inhibited by all three model strains viz. Pf-5, *Pf* CHAO and Q287. Rhizobial strain ST1 was inhibited by *P. fluorescens* Pf-5 but showed growth in presence of *Pf* CHAO, Q287 and G20. *R. leguminosarum 3841* and *M. loti* were found to be most sensitive to most of fluorescent *Pseudomonas* strains. *B .japonicum* and IC3123 exhibited growth in presence of all fluorescent *Pseudomonas* strains. Similarly IC3109 and IC3169 exhibited growth in presence of all fluorescent *Pseudomonas* isolates except G20 and *P. fluorescens* Q287, respectively. Fluorescent *Pseudomonas* strain G2 and G20 have shown inhibition to all rhizobial strains except IC 3109. The effect of partially purified metabolites on rhizobial strains was studied using ethyl acetate extracts of fluorescent *Pseudomonas* strains. *R. leguminosarum* 3841 was found to be the most sensitive rhizobial strain and inhibited by extracts of all fluorescent *Pseudomonas* strains except Strain C2. Rhizobial strain IC 3169 which got inhibited only slightly by DAPG that too at a higher

concentration, but exhibited inhibition by extracts of strains namely *Pf* CHAO, Pf5, Q287, G2, G13, G16, G45 and C7 in the plate assay.

Certain combinations of fluorescent *Pseudomonas* strains and particular rhizobial strain resulted in increased exopolysaccharide (EPS) production by the rhizobia. *Pf* CHAO induced more EPS production by *B. japonicum,* IC3123 and IC 3109 but not in other rhizobial strains. Triple culture technique to study the interaction of rhizobia, fluorescent *Pseudomonas* and *R. bataticola* showed that strains *Pf* CHAO, Q287 and Pf-5 show consistently high antifungal activity in presence of rhizobial cultures. Strain G2 and G1 has shown 35.5% and 22.22% fungal inhibition and also a strong inhibition of *R.leguminosarum*, while strain G38, G45 and G20 have shown fungal inhibition of 55.5%, 46.66% and 42% respectively but not the inhibition of rhizobial strains.

To conclude *R. leguminosarum and M. loti* can be considered as sensitive strains which upon co inoculation with fluorescent *Pseudomonas* strains may get inhibited and hence should not be used together as bioinoculants. Strains G20, G38 and G45 have shown strong antifungal activity but not a growth inhibition of any of rhizobial strains which could be due to a metabolite/concentrations which is inhibitory to fungal growth but not to rhizobial strains. G2 has shown both antifungal activity as well as inhibition of rhizobial growth so it may be concluded that the inhibitory molecule could be same for both inhibition.

Plant inoculations of *Vigna radiata* and *Cajanus cajan* by fluorescent pseudomonads incombination with other PGPR strains

Plant studies were performed with fifteen fluorescent *Pseudomonas* isolates on growth of *Vigna radiata,* with *Pf* CHA0 was used as positive control. Isolates C2, G2, G8, G16, G19, G20, G22, G26, G28, G31, and M3 have shown more positive effect on plant growth than *Pf* CHA0 while isolates G28, G35, G36 and G46 have shown effect equal to that of *Pf* CHA0. M3 showed the good effect on root while G8 on shoot. Seed germination assay under pathogen challenge was performed to check the biocontrol potential of fluorescent pseudomonad. Strains G8, G20, G26 have shown good capacity to control the infection of *R. bataticola* and protect *Vigna radiata* from infection. Model biocontrol strains *Pf* CHA0 has shown the biocontrol activity against *R. bataticola* but at relatively lower extent. Pf-5 and Q-287 did not shown effective plant protection biocontrol traits in plant assay.

Co inoculation effect of *Pf* CHA0, *E. asburiae* PSI3 and rhizobia on *Cajanus cajan:* In single treatments all three rhizobial isolates ST1, IC3123, IC3109 as well as *E.asburiae* PSI3 and *Pf* CHA0 showed positive effect on the growth of plant. However double combinations showed only slight better effect than the corresponding singlet counterparts while the triple combinations were found to have best effect.The root and shoot lengths and dry weights of above single, double and triple combinations were also increasing in the same order. Highest positive effect on root was observed in triple combination of PSI-3, Pf CHA0 and ST-1. The control pot which did not receive any pretreatment was showing least growth. Statistical analysis of pot result shows that positive effect of co inoculation was more on shoot than the root. Plant protection under biocontrol supportive medium, non-supplemented combination supported plant growth in uninoculated control and growth suppression of mung bean in *R. bataticola* treated set. *Pf* CHA0 has shown highest plant growth promotion out of all combination and MS medium control. The plant growth promoting effect of *Pf* CHA0 on *Vigna radiata* under biocontrol trait supporting showed that the combination with glucose, $FeSO_4$, KNO_3 and $ZnSO_4$ supplemented to MS medium and combination with glucose, citrate, nitrate, Pi, $ZnSO_4$, $FeSO_4$ and Mo^{2+} have shown inhibition of plant growth as compare to non-supplemented combination while inhibitory effect was minimized in inoculated with *Pf* CHA0. *Pf* CHA0 showed an effective inhibition of *R. bataticola* and plant protection in combination supplemented with glucose, $FeSO_4$, KNO_3 and $ZnSO_4$ supplemented to MS medium and combination with glucose, citrate, nitrate, Pi, $ZnSO_4$, $FeSO_4$ and Mo^{2+} as compared to un-inoculated control.

To sum up the major achievements work are as follows

- The present work has resulted in a collection of well-characterized novel PGPR strains of fluorescent pseudomonads from local soils and plants. Many of the strains are as effective or better in biocontrol action against plant pathogen *R. bataticola* as compared to standard strains reported in literature.
- An understanding of the factors controlling the levels of antifungal metabolite production in these strains vis-à-vis model strains reported in literature has shown strain to strain variation in many aspects. This approach can be used for developing strain specific

nutritional additives/amendments that can be effectively used in formulations to improve field performance.

- The finding that certain rhizobia are inhibited by antifungal metabolites produced by biocontrol strains is important to understand and effectively utilize the information about ecological interactions between different plant beneficial microorganisms and develop effective consortia.

- Plant inoculation experiments showed that combinations of multiple strains are more effective in plant growth promotion. Thus consortia with several different PGPR combinations can be expected to be better than single inoculants.