Conclusion

- Three different isolates MBAA1 (*Pseudomonas aeruginosa*), MBAA2 (*Bacillus cereus*) and MBAA3 (*Bacillus amyloliquefaciens*) were selected on the basis of their biocontrol potential from the 30 bacterial isolates isolated from rhizosphere soil of *Glycine max* and *Vigna radiata* of Sanand region of Ahmedabad, Gujarat.

- Isolates were characterized and screened *in vitro* for their biocontrol and PGP traits. Maximum phosphate solubilization was in MBAA2 (200.16 μg/ml), production of IAA in MBAA3 (69.2 μg/ml), Ammonia MBAA2 (32.25 μM/ml), siderophore production MBAA1 (24.39 mM/ml), chitinase, β-1,3 glucanase and cellulase production in MBAA1 (3.65 U/ml), MBAA1 (3.48 U/ml) and MBAA3 (25.64 U/ml) respectively. All isolates obtained were then tested by growing the strains with phytopathogenic fungi on dual culture agar plates.

- The maximum percent inhibition 91.14 % and 81.11% was observed *in vitro* with MBAA3 against *M. phaseolina* and *S. sclerotiorum*. In addition to these traits all the three isolates were further studied for their ability to reduce disease incidence in *Glycine max* and *Vigna radiata*. All the three isolates were found to reduce diseases caused by *M. phaseolina* and *S. sclerotiorum*.

- The were identified by 16S r RNA and the sequences were deposited in the GenBank nucleotide sequence database under the accession numbers KC662503 for MBAA1 (*Pseudomonas aeruginosa*), KF535139 for MBAA2 (*Bacillus cereus*) and KF535140 for MBAA3 (*Bacillus amyloliquefaciens*).

- These isolates were also studied for their growth profile to check its commercial viability, antibiotic sensitivity, utilization of carbon and nitrogen sources and Biochemical tests.
• MBAAT (T.citrinoviride) was selected from the fungal isolates on the basis of biocontrol potential and screened in vitro for their biocontrol and PGP traits like phosphate solubilization (121.51 µg/ml), production of indole acetic acid (IAA) (30.12µg/ml), ammonia (24.63 µM/ml), siderophore production (29.31mM/ml) and enzyme production like chitinase (6.18 U/ml), β-1,3 glucanase (4.93 U/ml) and cellulase (7.28 U/ml) production.

• Inhibition of phytopathogens by VOCs produced from T.citrinoviride MBAAT and was further confirmed by GCMS analysis. Highest hit and mass spectrum according to molecular weight reveled that they were 3-methylbutanal and 2-methyl, 2-butane which were detected at retention time 1.86 and 16.32 min.

• The selected bacterial and fungal isolates were further studied for their ability to reduce disease incidence in Glycine max and Vigna radiata and compared with commercial isolate CTC (Trichoderma viride). Treated plants showed vital biocontrol potential against M. phaseolina and S. sclerotiorum in the pot trials then the untreated plants.

• The next step was development of a suitable consortia that could be a feasible strategy for increased activity and better viability of plant growth promoting rhizobacteria (PGPR). When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only out competes with the others for rhizospheric establishments, but complement functionally for biocontrol traits.

• All the consortia were evaluated for the in vitro biocontrol potential and reduction of disease incidence in Glycine max and Vigna radiata caused by M. phaseolina and Sclerotina sclerotiorum. Glycine max plants treated with consortia
MBAA1+MBAAT (12.67%) showed lowest disease incidence followed by MBAA3+MBAAT (15.62%) in comparison to *M. phaseolina* infested plants (87%). The consortium MBAA1+MBAAT was found to be most effective in controlling disease in *Vigna radiata* against *M. phaseolina* (82.3%) that consider reducing disease incidence (18%).

- The survival of developed consortium prepared in carrier materials was assessed upto six months storage by spread plate technique and survival was measured in CFU/g or ml. MBAA1 + MBAAT followed by MBAA3 + MBAAT showed highest survival rate among the all consortia in charcoal followed by gypsum.

- Consortia MBAA1+ MBAAT and MBAA3 + MBAAT formulated in the charcoal and gypsum were used for pot experiments. MBAA1 + MBAAT possess highest survival rate in charcoal and this treatment is most effective in reduction of disease caused by *M. phaseolina* and *S. sclerotiorum* in *Glycine max* and *Vigna radiata*. Plants treated with MBAA1+MBAAT+ charcoal (15%) showed lowest disease incidence followed by MBAA1+MBAAT+gypsum (20%) and MBAA3+MBAAT+ gypsum (22.4%) in comparison to *M. phaseolina* diseased plants of *Glycine max*.

- These two consortia formulated in charcoal and gypsum were also used to study biometric parameters in field trials. The effect of treatment T1 (MBAA1+MBAAT+charcoal) showed 71.28% increase in the seedling vigor index against the control.

- This supported the *in vitro* findings of biocontrol potentials in the multi-species consortium. In the present investigation, the four isolates were studied with the possibility of a consortium, as effective bioinoculant formulation.
Maximum cellulase production (25.64 U/ml) was studied in MBAA3 and Placket-Burman experimental design was used to identify the significance of the ingredients of the media for the optimum production of cell wall degrading cellulase enzyme from MBAA3. The Response Surface Methodology was employed to study the interaction among the significant factors and also determine their optimal levels. The p-value suggested that the coefficient for the linear effect of CMC, MgSO4 and pH were most significant.

Three response surfaces were obtained by considering all the possible combinations. Validation was carried out under conditions predicted by the model. The optimal concentrations estimated for each variable were 1.84 g CMC, 0.275 g MgSO4 and pH 8.5. To validate the prediction of the model, additional experiments in triplicate were performed with the optimized medium. These experiments yielded the maximum cellulase activity of 30.62 U/ml.

The DNA was isolated from *B. amyloliquefaciens* MBAA3 and gene for cellulase enzyme was amplified. The amplification of 1500 bp gene of cellulase by primer set Cel F & CelR was obtained. The amplified product was sequenced and match with the available data in the NCBI and it was found the sequence of cellulase enzyme and it was submitted in the gene bank under the accession number KF929416.

The amplified cellulase gene was cloned in *E.coli* using cloning vector pCR™4-TOPOR and sequenced. The clone was expressed and the cellulase activity was determined 48.51 U/ml. The structure and function of cellulase protein from the clone was characterized by different bioinformatics tools.
Computational tools are useful for characterization of the relationships among protein sequence, structure, and function spaces. The recombinant *E. coli* strain showed higher cellulase activity (48.51 U/ml) in statistically optimizes media than the wild type (29.95 U/ml) which provide us the tools and understanding needed to make better use of these genes.