Conclusion

• The biofuel plant *Jatropha curcas* was selected and the rhizobial diversity was constantly monitored and studied for the species richness and evenness of PGPR, expecting such an empirical relationship from the single rhizosphere of *Jatropha* and its diversity in natural sites across Gujarat State was studied.

• Total 110 isolates from GS1, 98 from GS2, 35 from GS3, 76 from GS4, and 78 from GS5 were screened finally. Only those isolates which gave maximum growth, termed as fast growers, were selected for further studies. They were purified on their respective medium.

• Good diversity indices both Dominance indices (Example, Simpson index) and Information indices (Example, Shannon-wiener index) were studied to comprehend such diversity. It was advantageous to investigate the combined uses of species richness and diversity as well as to estimate the combinatorial effect of species richness and diversity in order to understand their role and distribution and function in their habitat.

• Results reveal the total heterogeneity of GS4 and GS5 and absence of equitability under the selected and defined condition. In the present study, GS4 is richer followed by GS1, while GS2 shows the least diversity of PGPR. Evenness index for Shannon index shows that whether there is same pattern of distribution of species or it varies while GS1 shows higher uniform distribution followed by GS3.

• These isolates were screened on the basis of various plant growth promoting traits like growth rate, phosphate solubilization, plant hormone production, biocontrol, HCN and siderophore production. As a result isolates (MS1, MS2, MS3, MS4 and MS5) showing best performances in all these parameters were selected for further studies on ACC deaminase production, antibiotic sensitivity, utilization of Carbon and nitrogen sources, FAME analysis, 16S rRNA and Biochemical tests.
Conclusion

- These isolates were also studied for their growth profile to check its commercial viability.

- All the five isolates *Brevibacillus brevis* MS1, *Enterobacter cloacae* MS2, *Bacillus licheniformis* MS3, *Micrococcus* sps MS4 and *Acinetobacter calcoaceticus* MS5 were suitable PGPR for the growth promotion of *Jatropha curcas*. Considering the plant growth promoting abilities of these five isolates for bioinoculant preparation is possible. This study show that these isolates having best characteristics of plant growth promoting potential that help in the seed germination, root and shoot length promotion and also increase the biomass of the plant *Jatropha curcas*.

- The nutrients enrichment of rhizosphere soil inoculated with these microbial inoculant attributed to the increased soil nutrient management process that contributes towards fertility status of the soil. Moreover, these isolates have positive impacts on soil characteristics and health necessary for better growth of planted biomass. This study showed the practical benefits of employing PGPR for a sustainable farming system and especially the three cultures considered best were MS1, MS3 and MS5.

- In another set of studies under stress condition was selected ACC deaminase as decreased levels of ACC result in lower levels of endogenous ethylene, which eliminate the potentially inhibitory effects of stress-induced higher ethylene concentrations. During last decade, understanding about the plant growth promoting rhizobacteria producing ACC deaminases has increased upto their molecular level leading to a number of commercial applications.

- *Enterobacter cloacae*, designated as MSA1 and *Enterobacter cancerogenus*, designated as MSA2 were recovered from the rhizosphere of *Jatropha* in the present work. This study first time confirms the ACC deaminase activity in the *Enterobacter cancerogenus*. 

218
Conclusion

- Several bacterial plant growth-promoting mechanisms were analyzed and detected like phosphate solubilization, Siderophore production, IAA production, GA₃ production and ACC deaminase activity in the isolated cultures.

- Isolates were grown until exponential growth phase to evaluate their ACC deaminase activity and the effect of pH, temperature, salt, metals and substrate concentration after the partial purification of enzyme by ion exchange chromatography.

- The Fourier transform infrared (FT-IR) spectra were recorded for the confirmation of α-ketobutyrate production. By using lineweaver Burk plot Km and Vmax value for ACC deaminase of both the organism was calculated in the different fractions. In this work, we discuss the possible implications of these bacterial mechanisms on the plant growth promotion or homeostasis regulation in natural conditions.

- *acdS* gene was PCR amplified from both the *Enterobacter* strains. After PCR, *acdS* gene of desired product was extracted and about 1.7 kb in size identical to each other was obtained for MSA1 and MSA2 both the strains. *acdS* gene coding for enzyme ACC deaminase can be a very useful candidate gene for the development of transgenics for abiotic stress management in plants.

- The ability of *Enterobacter cloacae* and *Enterobacter cancerogenus* to produce ACC deaminase, together with its inherent mechanisms to promote plant growth, may render this bacterium very useful in an agricultural setting.

- 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 plant growth promotion.
This is the first report regarding the growth promotion of *Jatropha curcas* by applying multi species consortia.

These symbiotic relationships are in fact beneficial in the global context, because they act to maintain ecological balance. Here we report cooperation between four rhizobacteria which belong to four distant genera established plant growth promoting bacteria due to their PGP ability. However they showed cooperation while growing together *in vitro*, which indicates their common ecological niche. All the four single organisms improved seedling growth.

The three isolates *Brevibacillus brevis* (MS1) + *Bacillus licheniformis* (MS3) + *Acienetobacter calcoaceticus* (MS5) have the ability to produce IAA, solubilize inorganic P, production of ACC deaminase and siderophore. They enhanced the growth of *Jatropha curcas* in individual trials. Besides, plant growth further improved maximally when three were applied together. Considering the plant growth promoting abilities of these four isolates, a non-specific, multi-species PGPC for bioinoculant preparation has been developed.

IAA production and siderophore increased about 45% in mixed-species culture, compared to maximum IAA and siderophore released in individual trials. The amount of phosphate solubilized was not affected. While the ACC deaminase production increased about 12% in mixed species culture.

Pot and field trials were conducted.

The highest germination of percentage (76.66 %) was observed in *Brevibacillus brevis* (MS1) + *Bacillus licheniformis* (MS3) + *Acienetobacter calcoaceticus* (MS5) and the germination capacity (83.33%) followed by *Brevibacillus brevis* (MS1) + *Bacillus licheniformis* (MS3) + *Micrococcus* sp. (MS4) + *Acienetobacter calcoaceticus* (MS5) (73.33 %) with germination capacity (80 %). The lowest germination percentage (63.66 %) was recorded from control treatment. Consortia help in the increased germination from control between the ratios of 5.26 to 21.04.
Conclusion

%.

Similarly the highest germination energy (32.77) was in MS1 + MS3 + MS5 followed by (31.52) in MS1 + MS3 + MS4 + MS5 and the lowest (28.75) was in control test.

- *Jatropha curcas* has a limited natural vegetative propagation and is usually propagated by seed. Besides, result of coinoculated seeds was better, where approximately four times higher shoot weight was recorded with respect to control in co-inoculated treatment. Similarly, 110% increase in plant length and 147% increase in grain yield was recorded. Data were higher with respect to control, as well as individual trials.

- This supported the *in vitro* findings of PGP 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.