# CHAPTER 6
# CONCLUSION

## 6. CONCLUSION:

Endophytic bacteria have fascinated considerable attention for their ability to promote plant growth through direct mechanisms or by acting as biocontrol agents. There is no single plant which is devoid of endophytic bacteria, instead a large population of bacteria are present inside the plant. Endophytic bacteria helps in mediating the medicinal properties of the plant. This thesis investigated the role of bacterial endophytes in growth promotion of *Achyranthes aspera* L. and its phytochemical properties.

A total of 73 endophytic bacteria were isolated from different five districts of Manipur. Of these, four isolates were selected based on primary screening of plant growth promoting activities. The morphological, biochemical and physiological characterization were carried out for tentative identification of the isolates. This was further confirmed by 16s rRNA sequence analysis. The selected isolates were identified as *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2.

A significant production of IAA was found in *P. aeruginosa* AL2-14B, *Serratia marcescens* AL2-16, *Serratia marcescens* AL6-10 and *Bacillus methylotrophicus* AST5-2. This production of IAA was influenced by L-tryptophan concentration and incubation period. 1.0 % concentration of L-Tryptophan was found to be optimum for IAA production by all the isolates except *B. methylotrophicus* AST5-2 where it was found to be maximum at 0.6% L-tryptophan suggested that these isolates have tryptophan dependent IAA production pathway.

Out of four selected isolates, three isolates viz, *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10 release siderophore in iron free succinate

medium which was confirmed by the instant decolourization of CAS reagent from blue to orange. The siderophore production was initiated after 24 hours of incubation during the growth phase. Synthesis of siderophore continued till the end of stationary phase and was reduced with initiation of death phase. It was evident that the siderophore production was high at late log phase, and amount of siderophore release was in accordance with the growth profile of isolates. Presence of catecholate and hydroxamate type of siderophore was given by *P. aeruginosa* AL2-14B, whereas both *S. marcescens* AL2-16 and AL6-10 indicated the presence of catecholate type of siderophore. Siderophore released was greatly influenced by media composition, iron concentration, carbon sources, nitrogen sources and organic acid sources. Succinic medium was found to be the best medium with maximum siderophore release for all the three isolates. Fructose was proved to be the best carbon sources resulting in appreciable amount of siderophore production in all the three isolates. Amongst nitrogen sources, urea proved to be the best utilizable nitrogen source for maximum siderophore production by *P. aeruginosa* AL2-14B whereas succinic acid was best nitrogen source for both *S. marcensens* AL2-16 and *S. marcescens* AL6-10. Malate was proved to be the best organic acid source resulting in appreciable amount of siderophore production by *P. aeruginosa* AL2-14B. In case of *S. marcescens* AL2-16, oxalate showed highest siderophore production. Whereas, in AL6-10, control (SM) served to be the best production of siderophore among other orgaic acids. Maximum siderophore production was observed at the concentration of 1µM of iron by *P. aeruginosa* AL2-14B and *S. marcescens* AL2-16. However *S. marcescens* AL6-10 showed its maximum production of siderophore at 0 µM of iron concentration.

All the four selected isolates solubilized tri calcium phosphate in Pikovskaya's agar, forming a clear halo around the colony after 48 h of incubation. From the result, it was found out that there is increased in phosphate solubilization by endophytes with the decreased in pH.

All the four selected isolates were able to grow on plates containing nitrogen-free medium. However, only three isolates viz, *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10 were able to produced ethylene by ARA test which indicated varied nitrogen fixation efficiency of different isolates. However ethylene production was not detected in case of *B. methylotrophicus* AST5-2. This was further confirmed by *nifH* gene amplification and desired amplicon of 781 bp corresponding to *nifH* gene was obtained by *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10.

In the present study, the colonization and re-isolation of all the four selected isolates from its host plant were carried out. The four potential isolates were re-isolated from infected seedlings of *A. aspera* L. In addition, the morphological and physiological characteristics of the endophytic bacterium recovered after experimental inoculations were indistinguishable from the colony morphologies of the inoculated organisms. All these isolates were not detected in leaves till 3DAI, however after 5 DAI, they were recovered from leaves as well as from stem of bacteria-treated *A. aspera* plants. The population size of the bacteria increases from its initial inoculation. Thus it was confirmed that these isolates were true endophytes.

*Achyranthes aspera* L. plants treated with three potential isolates (*P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10) individually showed

significant higher in shoot length, root length, number of leaves, fresh leaves weight, dry leaves weight, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, area of leaves as compared with un-inoculated control plants. Whereas there was no significant different found when plant was inoculated with *B. methylotrophicus* AST5-2.

The amount of total phenol content in the treated plant by *S. marcescens* AL6-10 was found to be higher than the control plant. However other three isolates treated plants gave lesser amount of phenol content than the control plant. *A. aspera* L. plant treated with all the four potential isolates showed higher DPPH radical scavenging activity compared to control plant. β-Carotene-linoleic acid assay of extract obtained from *P. aeruginosa* AL2-14B inoculated plant was found to be slightly increased than the control plant. Whereas the extract obtained from *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2 inoculated plants were found to be lower than the control plant. The reducing power of *A. aspera* L. leaves inoculated with *P. aeruginosa* AL2-14B and *B. methylotrophicus* AST5-2 were found to be higher than that of control plant. Whereas the reducing power of the extract inoculated with *S. marcescens* AL2-16 and *S. marcescens* AL6-10 were found to be slightly lower than the control plant.

This study emphasized on application of endophytic bacteria in the growth of *A. aspera*. Therefore, these isolates have potential to use as a plant biofertilizer or bioenhancer for plant growth improvement. Because endophytic organisms are an environmentally friendly alternative to chemical fertilizers and pesticides, it may be exploited as a growth promoting agent. Further, improved antioxidant activity in *A. aspera* L. is added advantage for the value addition of this medicinal plant.