Conclusion

Isolation and characterization of nitrogen fixing bacteria from wild legume haves never been reported from this region under study and the present study is the first from the state of Manipur, India. Studies regarding both legumes and rhizobia as well as legume-rhizobia interactions have not been fully documented from the region. Besides the studies about both free living as well as associative nitrogen fixer are not yet documented. As such the determination and categorisation of symbiotic, endophytic and rhizospheric nitrogen fixing bacteria from the region has a very prospective and worthwhile undertaking. In the present study more emphasis was given to the determination of the bacteria on their species and genus level through molecular characterization. Legume crops at least have been documented well by many worker all over the world but documentation of wild legumes and their interactions with bacteria still have a huge area to be explored. In the present study, fresh strain of symbiotic, endophytic as well as rhizospheric (associative) nitrogen fixing bacteria are isolated from the root nodules and rhizosphere soil of the source plants respectively. The isolated bacteria after preliminary examination through morphological, physiological as well as biochemical characteristics were screen for their nitrogen fixing ability through Acetylene Reduction Assay (ARA). The isolates showing positive readings in ARA were then subjected to NifH gene amplification to ascertain their nitrogenase activity. Finally incorporation of 16S rDNA gene amplification and sequencing in the study provided the information of the bacterial isolates upto their species level. During the study, isolation of *Enterobacter sp.* were more common of which many of the species were known nitrogen fixers. Isolation of *Beijerinckia, Pseudomonas* and *Bacillus sp.* further confirmed that the rhizosphere of wild legumes harbours a wide diversity of bacteria that are capable of nitrogen fixation. Isolation and identification very less known nitrogen fixing organism such as *Enterobacter hormaechei* and *Bacillus altitudinis* gives a very exciting prospect for future studies. Moreover, isolation of well known nitrogen fixing bacteria of the different *Rhizobium* species in the present study such as *Mesorhizobium huakuii*, *Neorhizobium huautlense*, *Bradyrhizobium japonicum* and *Mesorhizobium*

plurifarium provided a very positive aspect for future studies and researches. Although there are significant reports regarding the studies about the nitrogen fixing organism from around the world by many workers, both about free living and symbiotic, there is insufficient report of such research from the region. With the wide diversity of leguminous plants available in the region, huge scope is available for the such research to find out several bacteria associated with them regarding Biological Nitrogen Fixation. The process of biological nitrogen fixation being an eco-friendly approach towards increase crop productivity and soil enrichment is proving a good alternative to chemical fertilizer due to its safer and comparatively cleaner ways. Covering a broader area of sampling site and increasing the number of plant sources promises the possibility of finding out even more numbers of organisms capable of Biological Nitrogen Fixation.