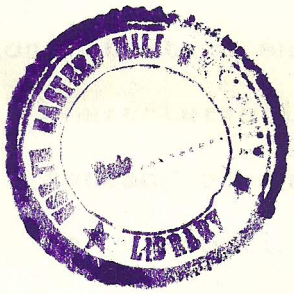
Study of Genetic Diversity of *Frankia alni* Strains Isolated from *Alnus nepalensis* Root Nodules found in Meghalaya

ABSTRACT

By

Ganesh G.



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The root nodules formed by actinomycete of *Alnus nepalensis* were of '*Alnus* type'. These nodules harboured *Frankia* symbiont which was isolated using various nutrient media. DPM was most suitable medium for the isolation and growth of *Frankia*. Four isolates obtained from *A. nepalensis* growing at various sites were chosen for the study.

The confirmation of these isolates as *Frankia* was carried out using morphological characters, nodulation tests and nitrogenase activity. The isolates showed characteristic features of *Frankia* by possesing thin hyphae, sporangia and vesicles typical of the symbiont. In addition to exhibiting nitrogenase activity, they nodulated seedlings of *A. nepalensis*. The results obtained therefore demonstrated the isolates to be *Frankia*.

Frankia isolates thus obtained were screened for genetic diversity. Measurements of hyphae and vesicles showed differences with AnpUS4 showing the largest diameters of hyphae and vesicles.

An absence of sporangia were found within the cortical cells of nodules thus classifying them as Sp^-.

In all the media used the isolates and strains had loosely arranged hyphae on 15 d and a network of hyphae had formed on 40 d. A difference in sporangial counts was observed with AnpST11 producing more than fifty sporangia per colony in all the media

on 40 d. The isolates showed different growth patterns on different media and all grew well in DPM. Utilization of carbon by AnpST11 was exceptionally well in acetate as sole carbon source while other isolates preferred propionate and pyruvate.

Nitrogenase activity varied in both *in vivo* and *in vitro* conditions. AnpUS4 was seen to be an efficient performer in *in vitro* conditions while AnpST11 did well during symbiosis. Addition of metals to the basal medium (DPM) enhanced the activity of nitrogenase to diiferent levels amongst isolates thus indicating the differences in their genetic composition. However, addition of nitrogen sources to the medium led to a fall in ARA rates, pointing towards the uptake of these sources.

Plasmids were detected in two isolates of *Frankia* from Upper Shillong (AnpUS4 and AnpUS8). Their molecular weights were around 20 Kb. However, it is unlikely that *nif* genes are present on these plasmids - firstly due to their size and secondly due to a similar behaviour by isolates not possessing the plasmids. The use of antibiotics as markers did not yield any significant differences. Neither any difference in the growth and nitrogenase activity of *Frankia* were seen.

Thus it can be concluded from the present study that the frankiae tested were genetically diverse. Variations not only existed amongst isolates but also with reference strains. However, differences narrowed down to nil values with a few parameters. For *in vitro* experiments AnpUS4 seemed to be a good choice and for studies of symbiotic nature AnpST11 was ideal. The

utility of AnpST11 would be established after field trials.