Journal of Phylogenetics & Evolutionary Biology

Evans, J Phylogen Evolution Biol 2015, 3:1
DOI: 10.4172/2329-9002.1000e111

Editorial Open Access

Phylogeny and Diversity of Rhizobial Bacteria

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Rhizobia are special bacteria that can live in the soil or in nodules formed on the roots of legumes. In root nodules, they form a symbiotic association with the legume, obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation. Several diagnostic tests were performed to compare the eight rhizobial isolates. Our results were broadly classified the native isolates as fast growing based on their growth on YEM media. The rhizobial isolates were shaped like short rods, as seen under the microscope. They measure 0.5 to 0.9 µm wide and 1.2 to 3.0 µm long (data not shown). Like most living things, they need a supply of air to live. The *Rhizobium* isolates were sensitive to kanamycin, Neomycin and Sulphemethooxazole antibiotics. There are three known determinants of bacterial permeability to an antibiotic: hydrophobicity, electrical charge, and amount of the antibiotic and the *Rhizobium* that showed a high level of resistance did not take up the antibiotics.

In order to visualize the ability of the isolates to solubilize different carbon sources, six different compounds were tested as a sole source of carbon. Five sugar sources tested namely; mannitol, sorbitol, sucrose, galactose and lactose and also, non-sugar source pectin was examined. All isolates were able to assimilate all carbon sources used and they showed growth responses to these sole carbon sources. However, mannitol was the best source of carbon source because it assimilated by all strains. On the other hand, pectin as hard carbon source (non-sugar source) was the hardest source to metabolism with isolates. At the same time, sucrose was the least carbon source (sugar source) utilized by isolates. Similar results have been reported before by.

It is well known that salt stress significantly reduces nitrogen fixation and nodulation in legumes. Deora and Singha have proposed that salt stress may decrease the efficiency of the *Rhizobium* legume symbiosis by reducing plant growth and photosynthesis, and hence nitrogen demand, by decreasing survival and proliferation of rhizobia in the soil and rhizosphere or by inhibiting very early symbiotic events, such as chemotaxis and root hair colonization, thus directly interfering with root nodule function. To date, some rhizobial isolates have been shown to grow under high salt conditions. In general, results of this research indicated that isolates were able to grow on 200mM of NaCl but they grew slowly on higher concentrations 300 mM. These results indicated that these isolates were relatively sensitive to the salt stress except two isolates Rl.2 and Rl.10. Similar observations were reported.

Despite that several genes have been identified in rhizobia response to salinity, the tolerance mechanisms of rhizobia to overcome salt stress remains unknown, mainly due to the fact that response and adaptation to salinity stress is a complex phenomenon involving many physiological and biochemical processes that likely reflect changes in gene expression. *Rhizobium leguminosarum* bv. viciae generally contains 1 to 10 plasmids which vary in size from 30 kb to more than 800 kb. Most of the genes required for nodule formation (*nod*) and nitrogen fixation (*nif* and *fix*) are carried on plasmids. Thus, loss of plasmids affected the relationship between *Rhizobium* and legume. Zurkowski isolated correlated plasmids from *R. trifolii* and they showed that Nod$^-$ mutants resulting from a prolonged treatment at high temperature were due to either loss of plasmid DNA or internal deletions in the plasmid. Zurkowski reported that at high temperature stress may cause the loss of the plasmid during cell division. Also, he added that the transfer of the plasmid into the Nod$^-$ isolates converted them to a Nod+ phenotype. Result of this research demonstrated extra plasmid with molecular weight about 23 Kb only in the two most salt tolerant isolates understudy. Therefore, it was concluded that salinity treatment may cause the presence of an extra plasmid in most native isolates. This result was in agreement with Maria.

The biodiversity of the indigenous isolates assessed by DNA fingerprinting method-RAPD-PCR further demonstrated a high level of genetic diversity among Faba bean rhizobial isolates. The eight primers were used to obtain RAPD fingerprint patterns because it has been shown to distinguish genomic variation within members of the same species of *Rhizobium*. From the analysis of eight faba bean isolates different RAPD fingerprint patterns were revealed. These fingerprint patterns varied in distribution according to the field site of isolation. Therefore, the population of bean-nodulating rhizobia in soils of Egypt was shown to be diverse, which allowed the selection of representative isolates for further analysis.

Thus, this result is further evidence that PCR-RAPD is a useful tool to conduct persistence and competitiveness studies in rhizobia. Also, it is in agreement with Rhitu et al. who tested 28 of indigenous rhizobia nodulating chickpea in India using RFLP to classify their isolates and El-Fiki who found that all RAPD primers detected one or more polymorphic DNA fragments among the studied rhizobia species and that RAPD is a very discriminative and efficient method for differentiating and studying genetic diversity of *Rhizobium*. Also, found that RAPD fingerprint patterns were able to distinguish genomic variation within members of the same species of *Rhizobium*.

Finally, from a phylogenetic perspective, legume root bacterial symbioses do not form a single monophyletic group, and different lineages within the α-proteobacteria are today recognized according to the systematic of Garrity et al. The phylogenetic analyses performed in this research confirmed the wide diversity of bacterial isolates associated to *O. ridentata*. The phylogeny of the 16S rRNA gene yielded two main clades. The first one grouped members of the *Rhizobiaceae* (*Rhizobium* and *Sinorhizobium*) and *Phyllobacteriaceae* (*Mesorhizobium*), with 100% bootstrap support, and the second clade brought together members of the *Bradyrhizobiaceae*, such as *Bradyrhizobium*, with full bootstrap support. The genus *Rhizobium* was grouped in a single cluster in which most of the sequences of *R. leguminosarum* isolates were placed. These results are similar with those obtained with Rincon *et al*. The classification of most isolates obtained after 16S rRNA sequence analysis was in a good agreement with classification obtained using RAPD data, however, 16S rRNA sequence analyses allowed better identification at species level.

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Received January 12, 2015; **Accepted** January 15, 2015; **Published** January 17, 2015

Citation: Evans AT (2015) Phylogeny and Diversity of Rhizobial Bacteria. J Phylogen Evolution Biol 3: e111. doi:10.4172/2329-9002.1000e111