RHIZOBIAL-FUNGAL-PHENOLIC INTERACTIONS IN N_2 FIXING SYMBIOSIS

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H. S. JAYASINGHE ARACHCHI

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ABSTRACT

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H. S. Jayasinghe Arachchi
Institute of Fundamental Studies
Hantana Road
Kandy
Sri Lanka

Phenolic compounds are a major group of natural chemical present in large quantities in the soil with variable structures and concentrations. Soil fungi constitute a major fraction of soil microbial biomass, influencing other microbes. Rhizobia interact with these natural chemicals and fungi which may affect the biological nitrogen fixation. Rhizobial growth and N_2 fixing symbiosis in the presence of soil phenolic compounds and fungi were examined in this study. This could help to understand the behavior of microbes in the soil.

Effects of different concentrations of four phenolic acids (protocatechiuc, *p*-coumaric, ferulic and vanillic) on population size of four rhizobial strains (*Bradyrhizobium elkanii* SEMIA 5019, *B. japonicum* TAL 102, TAL 620 and *Azorhizobium caulinodans* ORS 571) were studied. Further, the effects of phenolic acid affected rhizobia on N_2 fixing symbiosis of soybean were also investigated.

The effects of phenolic acids were concentration and structure dependant, and strain specific. The population size of TAL 102 increased when the culture medium was supplied with different phenolic acids as the sole carbon source. In many cases the presence of manitol in the medium masked the differential effects of phenolic acids on the population size. All four phenolic acids suppressed the population of TAL 620. Strain ORS 571 showed low population sizes at low concentrations followed by a growth recovery at high phenolic concentrations. All concentrations of ferulic acid increased the growth of SEMIA 5019 significantly. In different combinations, rhizobial growth and N_2 fixing symbiosis were negatively or positively affected by phenolic acids. Utilization of phenolic acids by rhizobia led to their biochemical changes, resulting in alteration in their symbiotic ability.

Based on the molecular weight, the possibility to fractionate different polyphenolic compounds present in diferent soils and plant materials without prior purification using one dimensional polyacrylamide gel electrophoresis was explored. Different polyphenolic compounds, extracted using 70% acetone were separated on 24% polyacrylamide gels (43% acrylamide and 3% bis-acrylamide). But, low molecular weight phenolics were not separated on the gel and retained at the bottom of the gel. This method provides a rapid fractionation of polyphenolic compounds according to their molecular weight. Further studies are however needed to fully establish the method.

Mycelial colonization of common soil fungi (*Aspergillus niger*, *Penicillium* spp. and *Mucor* spp.) by the rhizobial strains, resulting in the formation of bifilms was also studied. Further, the effects of fungal exudates and their secondary metabolites on the growth of rhizobia were evaluated. Bradyrhizobia gradually colonized the mycelia for 18 days, after which the biofilm structures collapsed with the release of rhizobial cell clusters to the medium. In general, there was no mycotoxin effects of the fungal exudates on the bradyrhizobial strains used, instead the strains utilized the exudates as a source of nutrition. This study indicates that the present microbial associations with the biofilm formation have important implications in the survival of rhizobia under adverse soil conditions devoid of vegetation. Moreover, it could have developed an as yet unidentified nitrogen fixing systems that could have contributed to the nitrogen economy of soils.

The effects of different concentrations of tannic acid on the rhizobial growth and on the soybean-rhizobial symbiosis were studied. Further, the effects of different concentrations of tannic acid on the fungal growth and fungal-rhizobial biofilms were examined. Population size decreased significantly in all the rhizobial cultures treated with different concentrations of tannic acid. Degradation products of tannic acid produced by the fungi were used as carbon and nutrient sources by the rhizobia.

Exudates of different fungi tested positively or negatively affected on the growth of different strains of the rhizobia. Rhizobial colonization on mycelia enhanced with the length of incubation time of the tannic acid treated co-cultures. Plant growth, nodulation and N accumulation of soybean were reduced when the rhizobial strains were affected by tannic acid.

Further studies are needed to unravel at molecular level, these alterations and their relatedness to N_2 fixing symbiosis and rhizobial persistence in the soils.