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Phenolic acids: Possible agents of modifying N_2-fixing symbiosis through rhizobial alteration?

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Abstract

Phenolic acids, low molecular weight phenolics, are precursors of a variety of antimicrobial compounds, root signalling molecules, and phytoalexins that play an important role in plant defence responses. In agro ecosystem, a large amount of litter is turned over during the cropping season, fallow period and land preparation. This releases a flush of phenolic acids, amounts of which exceed very much the quantities released in root exudation. In rhizobial inoculation of legumes, these phenolic acids, depending on the concentration, may affect the persistence of rhizobia in the soil and their symbiotic efficiency, in terms of N_2 fixation. The present study evaluates the effects of different concentrations of four phenolic acids (protocatechuic, *p*-coumaric, ferulic and vanillic) on population size of four rhizobial strains (*Bradyrhizobium elkanii* SEMIA 5019, *B. japonicum* TAL 102 and TAL 620, and *Azorhizobium caulinodans* ORS 571). Culture media with different concentrations of phenolic acids in the presence or absence of manitol were used to evaluate rhizobial population size on day 6. Rhizobial total proteins were extracted and electrophoresed on polyacrylamide gels. Further, the effects of phenolic acid-affected rhizobia on N_2 fixing capacity were also investigated by inoculating two of those strains to soybean. Phenolic acid-treated *B. elkanii* SEMIA 5019 and *B. japonicum* TAL 102 were inoculated to soybean, and plant growth, N accumulation and nodule dry weight were assessed in a pot experiment. The population size of TAL 102 was induced 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 rhizobial population size. All four phenolic acids used in our study suppressed the population size of TAL 620. Strain ORS 571 showed low population size at low concentrations followed by a growth recovery at high phenolic acid concentrations. Strain SEMIA 5019 treated with 0.03 mM ferulic acid produced the highest increase in shoot growth of soybean, (ca. 65%). Treating strain SEMIA 5019 with 9 m*M* protocatechuic acid produced the largest decrease in nodule dry weight (ca. 50%) without any significant changes in shoot N accumulation. *P*-coumaric acid, even at 0.12 m*M*, could stimulate the N_2 fixing activity of SEMIA 5019, whereas the same concentration reduced the effectiveness of TAL102 in a soybean-rhizobium symbiosis. Phenolic acid interactions with rhizobia led to biochemical, and hence physiological changes, resulting in an alteration in their symbiotic ability. Different leguminous plants secrete different phenolic compounds other than phenolic acids during root exudation. Further studies should therefore be conducted to evaluate the effects of those compounds on the symbiosis. It is concluded from this study that the effect of phenolic acids is concentration and structure dependant, and strain-specific. The effect will also be pH dependant. Thus, phenolic acids are possible agents for modifying the legume-rhizobial symbiosis.

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Introduction

The family of phenolic compounds is widespread in the plant kingdom, with a major source of phenolic compounds being lignin. Phenolic acids, low molecular weight phenolic compounds, bind the complex lignin polymer to the hemicellulose and cellulose in the plant cell walls (De Vries et al., 1997). They are involved in the cross linking of plant cell walls and are precursors of a variety of antimicrobial compounds, root signaling molecules and phytoalexins that play an important role in plant defense responses (Dixon and Paiva, 1995). A significant proportion of the phenolic fraction, some of it bound to protein or cell walls, remains within the leaf litter for some years (Harbone, 1997). Soil microorganisms rapidly decompose many of these compounds, but some appear to be stabilized against microbial degradation through sorption by organic matters, clay minerals and hydroxy aluminum or iron compounds in soil (McCarty and Bremner, 1986). In agro ecosystems, a large amount of litter is turned over during the cropping season, fallow period and land preparation. This releases a flush of phenolic acids, amounts of which exceed very much the quantities released in root exudation (Wu et al., 1998). In relation to rhizobial inoculation of legumes, these phenolic acids may affect the persistence of rhizobia in the soil and their symbiotic efficiency, in terms of N_2 fixation. Numerous studies have been conducted to investigate the utilization of phenolic acids as carbon sources by rhizobia (Irisarri et al., 1996; Stowers, 1985; van Rossum et al., 1995). The present study evaluates the effect of different concentrations of phenolic acids on the growth of four rhizobial strains. The phenolic acid affected rhizobia were protein-fingerprinted to examine their biochemical changes. Further, the effects of the affected rhizobia on N_2 fixing capacity were also investigated by inoculating soybean with two of these strains.

Materials and methods

Culturing of rhizobia

B. elkanii SEMIA 5019 and *B. japonicum* TAL 102, developed for soybean, TAL 620, a strain for chickpea, and *A. caulinodans* ORS 571 (Dreyfus et al., 1988), a stem-nodulating rhizobial strain of *Sesbania rostrata,* were used for the study. Bradyrhizobial cultures were maintained in yeast manitol broth (YMB) without agar whereas *A. caulinodans* cultures were maintained in peptone yeast broth (PYB) without agar (Somasegaran and Hoben, 1994). They were incubated on a rotary shaker at 28 °C for 6 days. Purity of the cultures was tested using yeast manitol agar with congo-red (CRYMA) plates and gram staining.

Table 1. Concentrations of phenolic acids applied to the rhizobial strains in yeast manitol medium

Phenolic acid	Concentration (m*M*)
Protocatechuic acid	12, 9, 6, 5, 2, 1.5
Ferulic acid	0.41,0.36, 0.31, 0.21, 0.11, 0.05
Vanillic acid	2.11, 1.84, 1.58, 1.32, 1.05, 0.53, 0.26
p-Coumaric acid	0.85, 0.75, 0.64, 0.53, 0.43, 0.21, 0.11

Application of phenolic acids

Four common phenolic acids found in the soils (ferulic, *p*-coumaric, vanillic and protocatechuic, Flaig, 1971; Whitehead, 1964) were used. Depending on their solubility in water, different concentrations of filter-sterilized phenolic acids (commercially available from Fluka chemie AG CH-9471, Buchs) were added to an autoclaved YMB in order to give the final molar concentrations indicated in Table 1. The range of these concentrations was within the range of phenolic acids found in soils (Guenzi and Mc Calla, 1966; Whitehead, 1964). The pH was measured in each medium. The four strains were cultured, as described above, and then subcultured in phenolic acid treated media. Three replicates were included for each treatment. Control cultures of each strain were maintained in YMB without phenolic acids. In addition, another set of cultures was maintained with a limited number of concentrations of each phenolic acid, but without manitol in YMB, in order to compare rhizobial population size when the acids were utilized as the sole carbon sources by the four strains. All the cultures were incubated at 28 °C on a rotary shaker and the experiment was carried out for 6 days.

Evaluation of rhizobial population size on day 6

Optical density of the bacterial cultures was measured at 600 nm using the UV-visible spectrophotometer (Shimadzu UV-1601). Barium chloride and sulfuric acid mixtures of McFarland's scale were used to cal-