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Extracellular polysaccharide-deficient mutants of Rhizobium strain CIAT899 induce chlorosis in beans

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Extracellular polysaccharide (EPS) production is required for nodulation by some rhizobia but not for nodulation of beans by *Rhizobium leguminosarum* bv. *phaseoli*. In order to study the role of EPS in nodulation of beans by the wide host range Rhizobium strain CIAT899, we produced EPS-deficient mutants of this strain.

Ten EPS-deficient Tn*5* mutants of CIAT899 from a total of six separate mutageneses were identified by screening for altered colony morphology on solid medium. The 10 mutants produced colonies that were 1/4 the diameter of colonies produced by the wild-type parent, CIAT899, suggesting that these mutants were either deficient in EPS production or grew more slowly than the wild type. In order to differentiate between these possibilities, growth rates and EPS production by the mutants was measured in broth culture. All but one of the mutants had doubling times similar to that of the wild type, approximately 2.0 h, but they all produced less extracellular material than the wild type.

All of the EPS-deficient mutants induced pink nodules on bean plants, but the plants developed a severe interveinal chlorosis on the trifoliate leaves. The chlorosis was not reversed by the addition of nitrogen or micronutrients to the plants. When each EPS-deficient mutant was applied to plants in a 1 : 1 mixture with the wild type, CIAT899 predominated in the nodules.

Our results suggest a relationship between the loss of EPS production and acquisition of the chlorosis-inducing phenotype since all of the EPS-deficient mutants also induced chlorosis. That the Tn*5* is causal to both mutant phenotypes remains to be demonstrated.

The phenomenon described here is similar to that described by LaFavre *et al.* (1988) who showed that capsule-deficient variants of *Bradyrhizobium japonicum* strain USDA 76 induce chlorosis on soybeans, whereas capsule-producing variants do not. Chlorosis induction by USDA 76 is due to production of rhizobitoxine, an amino acid derivative, which inhibits methionine metabolism. The similarity between the soybean system and the data presented here regarding plant symptoms and the effects of EPS suggests that the EPS-deficient mutants of CIAT899 may produce rhizobitoxine. This is the first indication of toxin production among the fast-growing rhizobia.

Reference

LaFavre J S *et al.* 1988 Can. J. Microbiol. 34, 1017–1022.