Presence of plant secreted flavones and expression of nodulation genes of rhizobium trifolii in acidic conditions.

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Sensitivity of the Rhizobium-clover symbiosis to low pH and associated acid soil factors have been outlined as possible contributing factors to reduced productivity of clover-based pastures on acid soils. Currently, we are investigating the affect of low pH on the early infection events in the formation of nodules on clover roots by acid tolerant and acid sensitive strains of R. trifolii.

The expression of Rhizobium nodulation (nod) genes that are involved in early recognition and induction of root nodules have been shown to be dependent on plant secreted flavones (e.g. 7,4'dihydroxyflavone) (1,2). The site of release of these compounds from the plant coincides with the location of the most infectable root hair cells (1), which usually occur behind the growing root tip. Furthermore, it has also been shown that for a rapid response to these flavones, the bacterium has to be in early logarithmic phase of growth (3).

Thus, two immediate possibilities arise which might explain why acid sensitive rhizobia are unable to initiate nodules under low pH conditions. Firstly, either the plant does not produce flavones under these conditions or alternatively the bacterium is unable to respond to the presence of flavone.

Materials and Methods

Regulation and expression of Rhizobium nod genes was determined by coupling the expression of the lac Z gene of E. coli to specific nod genes of R. trifolii (2), thus allowing nod gene expression to be measured by a simple in vitro technique (B-galactosidase assay). This nod/lac gene fusion has been introduced by conjugation into strains of R. trifolii with varying degrees of tolerance to acidity.

Exudates of subclover and white clover seedlings grown overnight in buffered solutions at pH's between 3.80 and 7.00 have been assayed for the presence of stimulatory flavones. Expression of nod genes in acid tolerant and acid sensitive strains of R. trifolii was determined over a similar range in pH.

Results and discussion

Results show that flavones are in fact released by clover plants at low pH that would otherwise prevent successful nodulation (although in some cases flavone was present in lesser amounts). Furthermore, the acid sensitive bacterium was unable to express nod genes in the presence of flavones at low pH's. In contrast, an acid tolerant strain of R. trifolii was able to express its nodulation genes at a lower pH than an acid sensitive strain. This correlates with nodulation studies of subterranean clover using an agar plate technique with acid tolerant and acid sensitive R. trifolii strains at defined pH's. We therefore suggest that the inability to initiate and perform the early stages of infection is largely a bacterial problem. We conclude that the ability to grow at low pH (acid tolerance) favours nodulation under such conditions in three ways: (i) these bacteria are able to establish logarithmic growth more rapidly under these conditions; it is at this growth stage that rapid nod gene expression occurs, (ii) they are then able to respond to the flavones that are released by the plant thereby initiating nod gene expression and (iii) the rapidity of these responses enables these bacteria to establish an infection on the most infectable root hair cells of the plant (the emerging root hair zone).

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