Competition Between Rhizobium Strains for Infection and Nodulation of Legumes

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Inadequate understanding of competitive mechanisms between Rhizobium strains for legume nodulation is a major obstacle to the greater exploitation of this nitrogen-fixing symbiosis in world agriculture. Replacement of indigenous soil rhizobia with more effective inoculant strains cannot yet be achieved with the required degree of consistency. However, recent advances in knowledge of early events in the basic infection process, such as the finding that legume flavonoids act as inducers of Rhizobium nodulation genes /REDMOND et al., 1986/, may now allow the interpretation of competition data at the biochemical/genetic level, thus revealing the mechanisms governing competitive relations between rhizobial strains.

In this paper data are presented to illustrate inter-strain variation in nodulating competitiveness of rhizobia and the reversal of the competitive relations of two rhizobial strains resulting from changes in legume rooting media. Methods for detecting the outcome of inter-strain competition by employing DNA-DNA hybridization techniques are also described.

Variation in nodulating competitiveness of Rhizobium trifolii strains

Several legumes are known to develop root nodules in a transiently infectible zone, just behind the root tip, bearing no visible root hairs /NRH zone/ at the time of first interaction with the free-living bacterial symbiont /BHUVANESWARI et al., 1981/. Studies with several strains of R. trifolii /STEPHENS and COOPER, 1988/ have shown marked variation in the speed of infection of this zone in white clover and data from two strains are shown in Fig. 1. By observing the appearance of nodules on a large sample of plants under high power microscopy it was established that by 2 days after single strain inoculation, 1186 had formed significantly more nodules than P3 in the region which was just behind the position of the root tip /RT/ at the time of inoculation. This region corresponds to the previously mentioned NRH zone. This feature was observed at two pH values in rooting solution: 6.7 and 5.0. When these two strains were presented to the host plant under competitive conditions /1:1 mixture/, 1186 formed a significantly higher proportion of nodules than P3 at both pH values /9:1 at pH 6.7 and 35:1 at pH 5.0/.

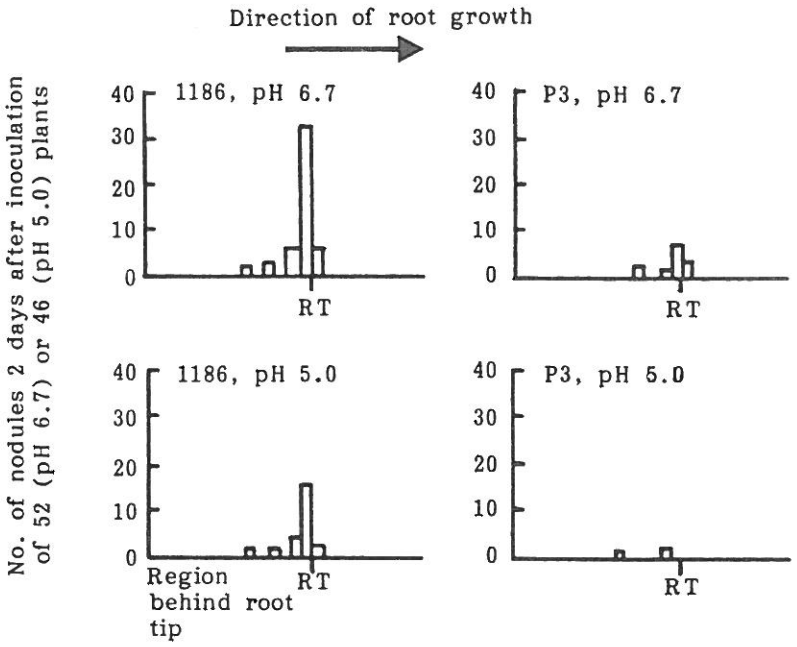

Fig. 1
Number and location of nodules formed by two strains of R. trifolii /1186 and P3/ at two pH values /6.7 and 5.0/ on white clover growing in rooting solution, 2 days after inoculation

The results demonstrate that a competitive advantage can be gained by strains of rhizobia which can initiate infection and nodulation quickly and efficiently. It may be possible to determine the reason for variation in infection rate by analysing strain properties such as susceptibility to flavone inducers, differential chemotactic responses and degree of binding to root hair primordia.

Reversal of competitive relations of paired rhizobial strains by changes in legume root environment

Studies with Lotus pedunculatus and its rhizobia /SOLAIMAN, 1983/ have shown that changes in the rooting medium of this legume can reverse the competitive relations of paired strains /Table 1/. In an agar rooting medium Bradyrhizobium strains CC814s and NZP2257 formed almost all nodules on a large sample of plants in competition /1:1 inoculum ratio/ with Rhizobium loti strain NZP2037. In liquid rooting solution, however, this result was reversed with R. loti strains NZP2037 and LP28 outcompeting Bradyrhizobium strain CC814s for nodulation of the host. Results from soil-grown plants were similar to those in agar rooting medium.

PARNISKE et al /1988/ showed that small changes in the root environment can alter the range of flavonoids synthesised by a legume and it is possible that this change in flavonoid spectrum could affect the competitive relations of rhizobia via an influence on nod gene induction or chemotaxis.

Table 1

Nodulating competitiveness of paired R. loti and Bradyrhizobium strains /1:1 inoculum ratio/ on Lotus pedunculatus growing in N-free agar rooting medium, rooting solution or peat soil at pH 4.5

Rooting medium	Strain combination /R.loti/Bradyrhizobium/	Number of nodules tested	% of nodules containing each or both strains	
Agar	NZP2037/CC814s	150	NZP2037	1
			CC814s	98
			both	1
	NZP2037/NZP2257	240	NZP2037	0
			NZP2257	100
			both	0
Solution	NZP2037/CC814s	54	NZP2037	100
			CC814s	0
			both	0
	LP28/CC814s	50	LP28	96
			CC814s	4
			both	0
Soil	NZP2037/CC814s	94	NZP2037	4
			CC814s	81
			both	15
	LP28/CC814s	54	LP28	18
			CC814s	78
			both	4

Detection of rhizobia in root nodules using strain-specific nucleic acid probes in conjunction with DNA-DNA hybridization

The outcome of most competition experiments for legume nodulation can only be determined by using identification methods which clearly discriminate between strains of a Rhizobium or Bradyrhizobium species. Recently, DNA hybridization technology has been applied to this particular branch of diagnostic bacteriology. HODGSON and ROBERTS /1983/ were the first workers to use total genomic DNA probes to identify R. trifolii strains by colony hybridization. COOPER et al. /1987/ also used total DNA probes to differentiate between R. loti and Bradyrhizobium spp. /Lotus/ in direct hybridizations of fluid from crushed nodules. Such probes are easy to prepare but their tendency to cross-hybridize with DNA from related strains limits their usefulness.

By using a procedure known as subtraction hybridization it is now possible to isolate strain specific fragments of DNA from rhizobia without resorting to cloning and screening of genomic libraries. The method involves repeated hybridization of prospective probe strain DNA to a mixture of DNA from related, cross hybridizing strains /subtracter DNA/ followed by the separation of unhybridized, and therefore probe strain-specific, DNA sequences from the subtraction matrix. Fig. 2 shows a schematic representa-

tion of a subtraction hybridization procedure /BJOURSON and COOPER, 1988/ which permits the rapid generation of specific probes from organisms for which no previous DNA sequence information is available.

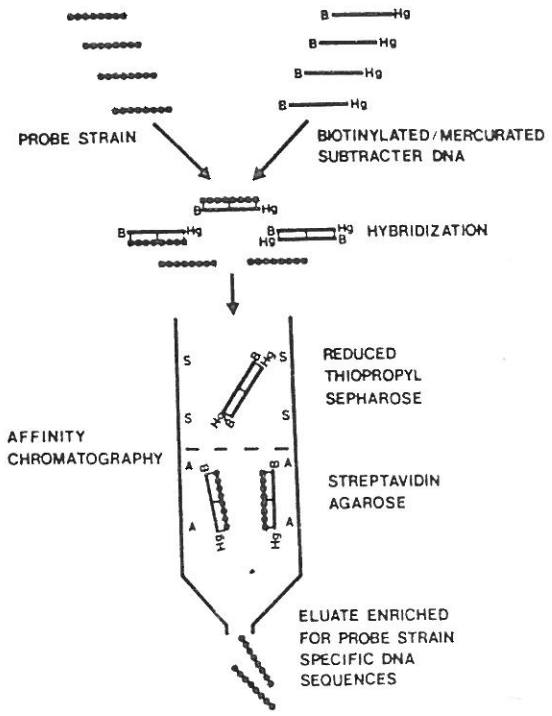

Fig. 2
Schematic representation of the use of biotinylated/mercurated DNA as a subtraction matrix for the generation of strain-specific sequences from the DNA of a related, cross-hybridizing bacterial strain

Summary

R. trifolii strains varied in the speed with which they infected and nodulated the "no root hair zone" of white clover. There was a positive correlation between the speed with which a strain nodulated this zone in single culture and its nodulating competitiveness in mixed culture. A change from solid to liquid rooting medium of Lotus pedunculatus was responsible for reversing the nodulating competitiveness of paired strains of rhizobia. Identification of rhizobial strains forming nodules in competition experiments can be achieved via DNA-DNA hybridization. Strain-specific DNA probes can be quickly generated by subtraction hybridization procedures which dispense with the need for screening genomic libraries.

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