
THE RHIZOBIUM COMPONENT OF THE NITROGEN-FIXING SYMBIOSIS

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Abstract

Rhizobia may be classified as either fast or slow growers, or alternatively into different cross-inoculation groups depending on the host plants they can nodulate. The clover, lucerne, lotus-lupin, and sainfoin cross-inoculation groups are of importance in New Zealand grasslands. Within each group there are numerous strains which differ in many respects, in particular in their ability to fix nitrogen with different host species. The clover and slow-growing lotus rhizobia are now widespread in New Zealand pasture soils but the others are not.

INTRODUCTION

ONE OF THE main reasons for sowing legumes in a pasture is their ability to fix atmospheric nitrogen. Nitrogen fixation occurs in nodules on the roots of these plants, and is a symbiotic process between the plant and its associated root nodule bacteria — the rhizobia. For nitrogen fixation to occur, rhizobial strains must be present which not only can invade the plant roots and form nodules, but can also fix nitrogen in these nodules. Such nodules are termed “effective”, and are pink inside when cut open, in contrast to “ineffective” nodules in which no nitrogen is fixed. These nodules are small and white inside, and are formed by rhizobial strains unsuitable for the particular host species. The same strains may, however, form effective nodules on another species.

Legume plants can grow perfectly well without nodules if there is plenty of nitrogen in the soil, and this may occur, for instance, in crops of peas sown for the first time in newly ploughed land, or in crops of French or navy beans, which are usually grown with added nitrogen fertilizers. In a pasture, however, legume seedlings in competition with grass will soon fail and die if they do not become nodulated with a rhizobial strain effective in fixing nitrogen. Also, the main purpose of having legumes in the sward is lost if they are not fixing nitrogen.

CHARACTERISTICS OF RHIZOBIA

Rhizobia are rod-shaped bacteria approximately 1 to 2.5 μm long. They are non-spore-formers, that is, they have no resting stage which is highly resistant to adverse environmental conditions. They are motile and are able to move in damp soil through the water films surrounding the soil particles, but the rate of movement by their own efforts is very slow (Hamdi, 1971). However, when water moves through soil it can carry rhizobia with it.

For their nutrition rhizobia require only simple organic compounds. They are stimulated by root exudates, particularly the root exudates of their host legumes, and are consequently more plentiful in the vicinity of these roots than in the bulk of the soil. In the absence of host roots rhizobial numbers fall and they may disappear altogether, although some groups of rhizobia can survive in soil for many years in the absence of their host plants.

CLASSIFICATION OF RHIZOBIA

The innumerable strains of rhizobia can be classified in various ways. Two of the most commonly used methods are related to (1) their speed of growth on artificial media, and (2) their ability to form nodules on different groups of legumes.

Classification according to their speed of growth on artificial media divides the rhizobia into two classes, the "fast growers"

TABLE 1: DIFFERENCES BETWEEN FAST- AND SLOW-GROWING CLASSES OF *RHIZOBIUM*

Property	Rhizobium Class	
	Fast-growing	Slow-growing
Growth rate (colony diameter after growth on an agar medium)	≥ 1 mm, 3-5 days	< 1 mm, 10 days
Gum production	+++ (free flowing)	+ (dense and sticky)
Acid production in culture	+	---
Utilization of sucrose*	+	---
Maximum tolerance ($\mu\text{g/ml}$) to antibiotics:		
Tetracycline	0.01-0.1	50-150
Vibramycin (doxycycline)	0.1-1.0	20-60
Viomycin	10-40	500-1500
Susceptibility to desiccation?	high	low
Ability to survive in acid soils	low	high

*Graham (1964).

†H. V. A. Bushby, pers. comm.

and the "slow growers" (Fred *et al.*, 1932; Buchanan and Gibbons, 1974). These two classes of rhizobia differ also in many other respects, and some of these differences are listed in Table 1. For example, the slow growers are more tolerant of several antibiotics, of desiccation and of infertile, acid soil conditions, while some fast growers appear more tolerant of alkaline conditions.

Classification of rhizobia according to the host legumes they can nodulate divides them into the so-called "cross-inoculation groups" (Fred *et al.*, 1932).

Three cross-inoculation groups, the clover, lucerne, and lotus-lupin groups, are of importance in New Zealand grasslands, and the sainfoin group is of some potential importance. Some characteristics of the rhizobia in these four groups are listed in Table 2.

CROSS-INOCULATION GROUPS OF RHIZOBIUM IN NEW ZEALAND PASTURES

CLOVER CROSS-INOCULATION GROUP

This is the most important cross-inoculation group of rhizobia in New Zealand pasture soils. It includes all those rhizobial strains nodulating true clover (*Trifolium*) species. All of these strains are fast-growing (Table 1), but they differ in many respects such as in their competitive and survival ability in soil (Chatel *et al.*, 1968). In particular, they differ in the *Trifolium* host species with which they will fix nitrogen. Strains highly effective in fixing nitrogen with one species are often only weakly effective or ineffective with another species. For example, many strains are effective in fixing nitrogen with white and red clover, but only a few of these are also effective with subterranean clover.

Since white clover is by far the most important clover in New Zealand, the rhizobial strains used in New Zealand clover inoculants are chosen for their high effectiveness and compatibility with white clover. These strains are poor nitrogen fixers with subterranean clover and this species thus requires a special inoculant. This requirement is further complicated by the fact that Woogenellup-subterranean clover is not nodulated freely by the rhizobial strain used in inoculants prepared for other subterranean clover cultivars, and a further special inoculant is required for Woogenellup. If other unusual clover species are sown they will usually require special inoculant strains. Examples are *Trifolium ambiguum*, of value in high country soil stabilization, and

TABLE 2: CHARACTERISTICS OF RHIZOBIA IN FOUR DIFFERENT CROSS-INOCULATION GROUPS

<i>Cross-inoculation Group</i>	<i>Host Species</i>	<i>Fast- or Slow-growing Rhizobia</i>	<i>Presence of Rhizobia in N.Z Soils</i>
Clover	true clovers	fast	very widespread
Lucerne	lucerne, other medicks, <i>Medicago</i> spp.	fast	present in some high pH soils
Lotus-lupin	lotus, lupins, serradella, gorse, broom	(fast {and slow	fast growers not present in most soils; slow growers widespread and very tolerant of acid soils
Sainfoin	sainfoin, crown vetch	fast	not present

T. semipilosum from the mountains of Kenya, which may possibly prove of value in the far north of New Zealand.

Clover rhizobia are widespread in soils throughout New Zealand, and are present as part of the soil micropopulation in all fertile cultivated soils and most grazed hill soils. However, their numbers in the soil may vary greatly over short distances. Thus, in typical North Island unimproved hill country there may be many clover rhizobia in soil under sheep camps and sheep tracks, but few or none detectable in soil of nearby slopes under moss, browntop, or manuka scrub. In areas where white clover is the main clover present, most rhizobial strains in the soil will be effective or moderately effective with white and red clover, but few effective with subterranean clover. In drier areas, however, where annual clovers are more plentiful, a greater proportion of the rhizobia in the soil are likely to be effective with subterranean clover.

LUCERNE CROSS-INOCULATION GROUP

The lucerne cross-inoculation group includes all rhizobia nodulating lucerne, the annual medicks, and *Melilotus* species. At present lucerne is the only host species for this group that is sown in New Zealand, and the rhizobial strains in lucerne inoculants are compatible with all lucerne cultivars sown at present. If, however, the current research interest in various annual medicks and *Melilotus* species for special areas leads to recommendations for the wider use of these plants, special inoculants may be required for them.

In contrast to clover rhizobia, the lucerne rhizobia do not thrive in most New Zealand soils (Table 2). This is largely because of their intolerance of acid soil conditions. In dry regions, or in limestone or coastal areas of New Zealand where the soils are not acid and where volunteer annual medicks or *Melilotus* species are found, lucerne rhizobia may be present as a permanent part of the soil micropopulation. Elsewhere, in more acid soils, they cannot be detected free in the soil. However, in stands of sown lucerne in slightly acid soils, the lucerne rhizobia can survive in and around the nodules and roots of the lucerne plants, but few if any are present in the soil away from the roots (Greenwood, 1965). Nevertheless, the inability of lucerne rhizobia to survive in acid soils is one major factor limiting the use of lucerne in these soils. A second limiting factor is the poor nitrogen-fixing ability of nodules produced on lucerne plants growing in acid conditions. These nodules, which are

formed only after massive inoculation of the plants with Lucerne rhizobia, are large and few in number. The reason for their poor nitrogen-fixing ability is not clearly understood.

LOTUS-LUPIN CROSS-INOCULATION GROUP

The lotus-lupin cross-inoculation group is more heterogeneous than the previous two groups. First, it includes rhizobia nodulating a more diverse range of host plants, including lotus and lupin species, serradella (*Ornithopus* spp.), broom (*Cytisus* spp.), and gorse (*Ulex europaeus*). Secondly, in contrast to the clover and lucerne cross-inoculation groups which contain only fast-growing strains, it contains both fast- and slow-growing rhizobia (Table 3). Thus the strains which nodulate and fix nitrogen best with *Lotus corniculatus* and *L. tenuis* are fast growers, but all other host species which are of importance in New Zealand at present (*Lotus pedunculatus* and annual *Lotus* spp., lupins, serradella, gorse and broom) are normally nodulated, and for the most part fix nitrogen, only with slow-growing strains. There are, however, a few fast-growing strains which can fix some nitrogen with *L. pedunculatus* and annual *Lotus* spp. The lotus hybrid, 'Grasslands 47 12' (*L. pedunculatus* (tetraploid) x *L. corniculatus*), is somewhat variable in rhizobial response though closer to the female parent, *L. pedunculatus*. The recommended inoculant for this hybrid is a mixture of two strains — a slow-growing strain effective with *L. pedunculatus*, and a fast-growing strain effective with *L. corniculatus* and moderately effective with *L. pedunculatus*.

TABLE 3: EFFECTIVENESS OF RHIZOBIUM WITHIN THE LOTUS-LUPIN CROSS-INOCULATION GROUP

Rhizobium Type	Host Species			
	Lotus corniculatus	Lotus pedunculatus	Grain Lupins	Gorse and Broom
Fast-growing (many strains)	E, ME	I	0	O, I
Fast-growing (a few strains)	E, ME	ME	O, I	I
Slow-growing (many strains)	J	E, ME	E, ME	E, ME
Slow-growing (many strains)	I	I	I	E, ME

* E = effective

ME = moderately effective

I = ineffective

0 = non-nodulating

Although *L. pedunculatus*, lupins, serradella, gorse, and broom are all nodulated by slow-growing rhizobia and there are many strains which fix nitrogen with all of them, the strains which fix nitrogen best with *L. pedunculatus* are not the best with the crop lupins or serradella. There are also many strains able to fix nitrogen with gorse and broom which are quite ineffective with *L. pedunculatus* and the crop lupins (Table 3). However, rhizobia under stands of gorse or broom normally include some strains effective with *L. pedunculatus*.

Fast-growing rhizobial strains effective with *Lotus corniculatus* have not been detected in New Zealand soils except from those few areas where *L. tenuis* or *L. corniculatus* have become naturalized. In contrast, slow-growing lotus rhizobial strains are very widespread in New Zealand soils, though not as widespread as clover rhizobia (Table 2). They are, however, able to survive in some soils which are too acid to support clover rhizobia.

SAINFOIN CROSS-INOCULATION GROUP

The boundaries of this group have not, as yet, been clearly defined. There is, however, considerable effective cross-nodulation between sainfoin (*Onobrychis viciifolia*) and crown vetch (*Coronilla varia*). There is also some nodulation, largely ineffective, of sainfoin by certain rhizobial strains from *Carmichaelia* and some other indigenous legumes. Rhizobia effective with sainfoin are not present in New Zealand agricultural soils, and from the small amount of work done so far it appears that sainfoin rhizobia are at least as sensitive as lucerne rhizobia to soil acidity.

DISCUSSION AND CONCLUSIONS

Apart from sainfoin, none of the legumes referred to in this paper will nodulate with rhizobial strains from indigenous legumes, and it can be assumed that all the rhizobia which now nodulate them in New Zealand have been introduced, mainly unintentionally and during the early days of settlement. They will have been spread around the country and across farms in animal feeding stuffs, seed and lime, on agricultural implements, the feet of animals, and in dust and flood waters. The effectiveness of these methods is exemplified by the wide distribution of the clover and slow-growing lotus rhizobia in New Zealand soils. In some soils they may now be detected in small numbers beyond the range of their host plants. However, it is unwise to assume rhizobia are present. Some soils contain a microflora

antagonistic to rhizobia, and in these soils it is particularly difficult to get them established. Lucerne and sainfoin should always be inoculated, as should other legumes sown in areas where the same species has not grown for several years, unless trials have shown that good nodulation is obtained with uninoculated seed and that no response is obtained from inoculation.

Although inoculation of the seed is not the only method of introducing rhizobia into a soil, it is the method almost always used. It has **the** advantage that the rhizobia are well placed just above where the roots of the young seedlings will develop, and thus they do not **have** to move far through the soil to form **the first** nodules. However, they do **have** to move a short distance, and this is of particular significance where the seed is sown from the air, and may lodge at or above the soil surface. Rain soon after sowing is of benefit in that it will wash some of the rhizobia into the soil, and **in fact** some rain may be essential in order to get good nodulation from such sowings.

Another factor to remember is that the seed surface is not a natural habitat for rhizobia and they will tend to die once they are inoculated on to seed. Their rate of dying depends on a number of factors. High temperatures and cycles of wetting and drying are particularly adverse. Coating the seed with finely ground Gafsa phosphate and dolomite or other suitable material, together with a suitable sticker, may reduce the rate of death if well carried out, and will certainly **help to** protect the rhizobia if the seed is mixed with superphosphate before sowing.

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