ACTIVITIES OF SOME RHIZOSPHERE BACTERIA IN WAIKATO PASTORAL SOILS

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Microorganisms associated with plant roots may be beneficial or harmful for plant growth. The well known beneficial associations include legume-rhizobia interactions and root-mycorrhizal fungi interactions. Other, lesser known beneficial associations include bacteria and fungi which produce antibiotics in the rhizosphere of plants which inhibit root pathogens. Inhibitory or deleterious effects of microorganisms mainly result from pathogens invading root tissues or microorganisms which are not pathogenic but produce compounds which adversely affect seed germination and root health. One of the inhibitory compounds produced by some rhizosphere bacteria is cyanide, which is a powerful respiration inhibitor. Some plants such as white clover (*Trifolium repens* L.) are also able to produce cyanide when tissues are damaged, and this is thought to be a defence mechanism against some plant pests. It is not known whether cyanogenic plants can tolerate the bacterial cyanide to a greater extent than non-cyanogenic plants.

TABLE 1: Germination scores¹ for different cultivars of white clover grown at different cyanide concentrations.

Cyanogenic status	Cultivar	Cyanide concentration				
		100µM	50µM	25µM	12.5µM	0µM (Control)
Low	Lena	2	2	6	5	6
	NFG (Gigant)	2	4	5	5	6
	Lodigiano (Gigant)	2	3	6	6	6
Medium	Milkanova	2	5	6	6	6
	Rivendel	2	3	5	5	6
	Sandra	2	1	5	5	6
	Lirepa	2	2	5	5	6
High	Pitau	5	n.d.	5	5	7
	Huia	2	2	4	4	6
	Olwen	2	3	5	5	6
	Sabeda	1	3	5	5	6
	Ross	2	3	5	4	6

¹ Scores indicate an average visual assessment of germination status.

1 - Imbibed seed coat cracked; 2 - Radicle visible; 3 - Radicle 0-5 mm;
4 - Radicle with root hairs; 5 - Seed leaves exposed; 6 - Shoots < 20 mm;
7 - Shoots > 20 mm n.d. = not determined

Rhizobacteria were isolated from permanent pastures in the Waikato region. Turves were brought to the laboratory, the roots were washed free from soil, and

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ryegrass (*Lolium perenne* L.) and clover roots were separated. Measured quantities (usually 1 g) were shaken vigorously with 2 g of fine glass beads in 9 ml of sterile distilled water. The resulting suspension was further diluted and plated on media for bacterial counts from 'washed roots'. The roots were separated from the glass beads and ground to a suspension in 9 ml of water. This was diluted and plated to obtain counts from 'ground roots'. Media used were trypticase soy agar (TSA) to obtain total counts and King's medium B (KB) to obtain fluorescent *Pseudomonas* counts.

Isolates were tested for their ability to inhibit pathogenic fungi *in vitro*. The pathogenic fungus was spot plated on potato dextrose agar (PDA) and after 24-48 h the bacterium was streaked as a line on each side of the fungal colony. After 3-6 days incubation, the inhibition of fungal growth (if any) was measured. Ability to produce cyanide was determined according to Bakker and Schippers (1987). To determine the effect of rhizobacteria on seed germination, seeds of white clover cultivars with different cyanogenic status were surface sterilised and approximately six seeds were placed on a sterile filter paper in a petri plate. A 24 h culture of the bacteria was scraped and cells suspended in distilled water to obtain approximately 10^7 cells per ml. Two ml of the culture was added to each petri plate, sealed with plastic film and incubated in the dark at 20°C for 5-7 days. Germination and growth of seedlings were scored per plate as given in Table 1. Each experiment was repeated at least once.

Effect of cyanide on seed germination was determined by placing white clover seeds in water agar containing cyanide concentrations ranging from 0 - 100 µM, sealing the petri plates and incubating at 20°C in the dark. The pH of agar was approximately 6.8. Germination was scored as in Table 1.

TABLE 2: Percentage of fluorescent *Pseudomonas* spp. isolates from ryegrass and clover roots producing cyanide and exhibiting antifungal activity.

	Cyanide production		Antifungal activity	
	Ground roots	Washed roots	<i>F. oxysporum</i>	<i>P. ultimum</i>
Ryegrass	78.1	62.5	17	44
Clover	83.1	82.9	8	30
SED		8.0		

The production of cyanide by rhizobacteria can be detrimental to plant growth (Schippers *et al.* 1987). The germination of all clovers except 'Pitau' was inhibited in the presence of this compound (Table 1). There was no relationship between cyanogenic status of clover and the degree of inhibition of germination from externally applied cyanide. More ($P < 0.05$) fluorescent *Pseudomonas* spp. from washed clover roots were capable of producing cyanide compared to isolates from washed ryegrass roots (Table 2). There was no significant difference between cyanide producing *Pseudomonas* spp. isolated from ground ryegrass and ground clover. It is not known if cyanide production by rhizosphere bacteria can cause detrimental effects on clover or grass growth in field conditions.

Many investigators have found rhizosphere bacteria with the potential to inhibit or reduce plant growth (Astrom 1991). These bacteria are assumed to produce chemicals that affect root cell permeability or affect other physiological processes in roots (Schippers *et al.* 1987). In one of our trials, up to 14% of rhizobacteria isolated from ryegrass roots were able to inhibit root growth in germinating seedlings of ryegrass and white clover. Preliminary identifications show that these belong to the genera *Klebsiella*, *Enterobacter* and *Pseudomonas*. These groups of organisms have been found to inhibit the growth of other crops such as sugar beet (Suslow and Schroth 1982). The inhibitory activities of these bacteria are not due to cyanide, but are most likely due to production of low molecular weight toxins (Bolten *et al.* 1989). Work is

continuing to determine the effects of cyanide-producing rhizobacteria on seed germination and plant growth.

Rhizobacteria also play an important role in natural biological control processes in the root zone by inhibiting the growth of pathogenic fungi. Our results suggest that a proportion of fluorescent *Pseudomonas* spp. are able to produce compounds that inhibit the growth of the two test pathogens, *Fusarium oxysporum* Schlecht. and *Pythium ultimum* Trow under laboratory conditions (Table 2). If this antagonism towards pathogens also exist in the rhizosphere, fluorescent pseudomonads may be having considerable beneficial effects on pasture growth.

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