# CHEMOTACTIC MOVEMENT OF RHIZOPLANE BACTERIA TOWARDS ROOT EXUDATES OF WHITE CLOVER AND RYEGRASS

S.U. SARATHCHANDRA and J.A. BROWN

*AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton*

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Motility is an important attribute for many bacteria to survive in competitive environments. Competition in soil results in some bacteria preferentially moving towards nutrient-rich regions (Chet and Mitchell 1976). One of the most nutrient-abundant regions of the soil is the root surface of actively growing plants which continuously secretes amino acids, sugars and other nutrients sought by microbes. Chemotaxis is an important mechanism in the colonisation of microorganisms in plant roots by microorganisms (DeWeger *et al.* 1987).

Most perennial pastures in New Zealand are a semi-permanent long-term dual culture of white clover (*Trifolium repens* L) and ryegrass (*Lolium perenne* L). Root systems of these intertwine in a common 'rhizosphere'. Carbon (C) rich root exudates provide the major energy source in the rhizoplane for microbial growth. There is little or no information relating to saprophytic bacterial colonisation of white clover and ryegrass root surfaces or specific affinity towards host plant root exudates. This experiment was to determine the chemotactic movement of bacteria isolated from pasture rhizoplanes towards seedling exudates of ryegrass and white clover.

Thirty-five strains of fluorescent *Pseudomonas* spp. were selected (Sarathchandra *et al.* 1993) of which 16 were isolated from ryegrass roots (ryegrass pseudomonads) and 19 from white clover roots (clover pseudomonads). The *Pseudomonas* spp. used were highly motile and were grown for 24 h to ensure maximum motility.

White clover (cv. Kopu) and ryegrass (cv. Yatsun) seeds were surface sterilised, checked for sterility by plating on 1/4 strength potato dextrose agar (PDA). Approximately 500 of these contaminant-free seeds were put into a sterile 500 ml flask containing 5 ml of distilled water and incubated in an orbital shaker for 24 h at 20 °C in the dark. The liquid in the flask was siphoned off to remove nutrients carried over from PDA and checked for contamination. Five ml of sterile distilled water was added to the seeds and incubated for a further 48 h under artificial illumination. By this time the seeds had germinated, the radicle and the seed leaves (cotyledons) had emerged. The solution containing exudate was siphoned off and frozen (-20 °C) for later use.

The *in vitro* chemotactic activity of bacteria was determined as described by Scher *et al.* (1985). Bacteria were grown in King's B (KB) medium for 24 h at 25°C. A loopful of the culture was suspended in 10 ml of sterile 0.1M $MgSO_4$ solution and the optical density at 580 nm was adjusted to between 0.65 and 0.85. Capillary tubes (1 µl) were heat sealed at one end and dipped in 1 ml of the clover or ryegrass seedling exudate. Controls consisted of capillary tubes dipped in 0.1M $MgSO_4$ solution. Tubes were then filled with the exudate or the $MgSO_4$ solution. These tubes were then rinsed and placed in Universal bottles containing the bacterial suspension pre-warmed to 25°C. Three capillaries containing white clover exudate, ryegrass exudate and the control were put into each of the bacterial suspensions and incubated at 25 °C for 1 h. Capillaries were then rinsed with 95% ethanol followed by sterile water. The sealed ends were broken and the contents of each capillary serially diluted, plated on KB medium and counted after 48 h incubation. The assay involving each bacterial strain was replicated three times.

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$Log_{10}$ transformed data (bacterial numbers) were analysed using standard analysis of variance methods.

Ryegrass pseudomonads tended to have a greater affinity towards ryegrass seedling exudates and clover pseudomonads a greater affinity towards white clover seedling exudates while the controls attracted the lowest number of bacteria (Table 1). Opposing trends without the inclusion of the controls were significant ($P<0.05$). Two of the 35 strains did not migrate into control ($MgSO_4$) capillaries.

It has been hypothesised that the migration of bacteria towards root exudates is a general phenomenon resulting from their continuing search for nutrients. Present observations that greater numbers of pseudomonads, irrespective of their origin, migrated into seedling exudates compared to the $MgSO_4$ control confirm this. In addition, the results of the present work also suggest the possibility that bacteria which are normally resident on rhizoplanes of white clover or ryegrass may have adapted to thrive on the nutrients contained in the root exudates of their specific host plant and, given the opportunity, may preferentially colonise the roots of their host plant. This is an interesting observation considering the nature of the intertwined root systems of the white clover/ ryegrass pastures of New Zealand.

**TABLE 1: Chemotaxis of fluorescent *Pseudomonas* spp. from white clover and ryegrass roots towards white clover and ryegrass seedling exudates and $MgSO_4$ controls.**

| Exudate type | White clover pseudomonads[1] (log10 bacteria / ml) | Ryegrass pseudomonads[2] ($log^{10}$ bacteria / ml) |
|---|---|---|
| White clover | 5.65* | 5.56* |
| Ryegrass | 5.61* | 5.65* |
| Control | 5.29 | 5.42 |

[1] Mean for 19 strains isolated from white clover roots
[2] Mean for 16 strains isolated from ryegrass roots.
* The trends were significant at 5% when controls were omitted from the analysis.

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