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Chemotaxis by *Rhizobium meliloti*

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Summary. Chemotaxis by *Rhizobium meliloti* strain Ve 26 has been studied and conditions required for chemotaxis have been defined, using the Adler capillary assay technique. Several sugars and amino-acids were shown to be attractants with varying effectiveness for this organism: sugars are weak attractants (except gluconate) and amino-acids are good attractants (except unpolar amino-acids).

Key words: Chemotaxis – *Rhizobium* – Amino acids – Sugars

Many studies have been conducted to elucidate the mechanism of infection and specificity of symbiotic associations between *Rhizobia* and legumes. Since *Rhizobia* are motile bacteria, it has been suggested that motility could extend their range in the legume rhizosphere. Furthermore, positive chemotaxis exerted by some substance exuded by plant roots could play a specific role in this association (Currier and Strobel 1976; Currier and Strobel 1977a). Although motility and chemotaxis function are not absolutely necessary *in vitro* for infection and nodulation processes, they may provide advantages to strains possessing them (Ames et al. 1980; Napoli and Albersheim 1980). Our purposes were to determine conditions for motility and chemotaxis studies, by the Adler capillary assay (Adler 1973), and then to test the chemotactic behaviour of a fast growing *Rhizobium meliloti* strain, toward simple molecules.

Materials and Methods

Rhizobium meliloti strain Ve 26 is a wild strain isolated by Obaton (Station de Recherche de Microbiologie du Sol, Dijon, France). This strain was stored on slants of Wright medium (Wright 1962). The L-amino-acids were obtained from Sigma and D-sugars from Merck. To determine growth and motility rich liquid medium "TY" (Beringer 1974) and minimal liquid medium "M_9A" (Ucker and Signer 1978) were developed. For chemotactic assays, we used both plate and capillary methods with the basic medium "C.W." described by Adler (1973) was that defined by Adler (10^{-2} M potassium phosphate buffer, 10^{-4} M potassium ethylenediaminetetraacetate). The mean accumulation (at least in triplicate assays) was expressed in terms of total cells per capillary. The maximum range for the average was 27%. To normalize the differencies in motility from day to day the chemotactic response was also expressed as the ratio (R) of the accumulation in the attractant capillary to that in control capillaries. This value showed less variability between different cell preparations than did the absolute accumulation. A relative response of 2.0 or greater was considered to be significant.

Results and Discussion

Observations on swarm plates or in phase-contrast microscopy showed, whatever the medium we used, our bacteria were motile. Motility consisted of straight line movements interspersed with periods of tumbling. Rigid complex flagella break easily and two washed affected the motility of the *Rhizobia* population. We have established on lysine data the standard conditions for *Rhizobium* chemotaxis: bacteria harvested in the exponential phase were washed once in the medium C.W. pH 7 and used at a concentration of 6×10^7 per ml; chemotaxis assays were run at 30° C. However long the assay time, over the range studied (15–90 min), the number of *Rhizobium* in the capillaries is maximal and constant: we have choosed 60 min for practical reasons like other authors. Tables 1 and 2 list various sugars and amino-acids for their ability to attract *Rhizobium meliloti:* threshold is determined by extrapolating a double-logarithm plot of response versus chemical concentration to the background "base-line"; peak concentration is the concentration for a chemical in the capillary which gives the largest response on the concentration-response curve; peak response is expressed by the ratio (R) at the peak concentration. Those data show that the best attractant, if we consider the chemotactic ratio, is aspartate ($R = 54$); but the threshold ($4.5 \cdot 10^{-7}$ M) is higher than others (about 10^{-7}–10^{-6} M). Generally, amino-acids with an acid or basic character are stronger attractants.

Table 1. Chemotaxis of *Rhizobium meliloti* toward some sugars. Cells were grown on "M_9A" (0.2% w/v fructose). Each compound was tested at concentration up to 0.25 M

Sugars	Threshold (M)	Peak concentration (M)	Peak response (ratio: R)
L-Arabinose	1.6×10^{-5}	>0.25	4.5
2-Keto-gluconate	8.4×10^{-6}	>0.25	3
D-Fructose	1.07×10^{-4}	>0.25	3
Gluconate (Na)	7.2×10^{-6}	>0.25	24
D-Glucosamine	7.2×10^{-5}	>0.25	1.6

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Table 2. Chemotaxis toward amino-acids. Cells were grown on "M_9A" (0.2% w/v fructose). Each compound was tested at concentration up to 2.5×10^{-2} M. *ND* Not determined

Amino-acids	Threshold (M)	Peak concentration (M)	Peak response (ratio: *R*)
Alanine	1.7×10^{-6}	$>2.5 \times 10^{-2}$	4.40
Asparagine	ND	ND	ND
Aspartate	4.5×10^{-7}	$>2.5 \times 10^{-2}$	54
Arginine	2.3×10^{-7}	2.5×10^{-3}	10.7
Cysteine	7.5×10^{-7}	2.5×10^{-3}	9
Glycine	1.2×10^{-7}	2.5×10^{-3}	5.4
Glutamate	5.6×10^{-7}	$>2.5 \times 10^{-2}$	5.8
Glutamine	ND	ND	ND
Histidine	1.3×10^{-6}	2.5×10^{-3}	9.9
Leucine	5.5×10^{-7}	$>2.5 \times 10^{-2}$	3
Lysine	2.2×10^{-6}	2.5×10^{-3}	17.7
Methionine	2×10^{-7}	2.5×10^{-4}	5.90
Ornithine	1.2×10^{-7}	2.5×10^{-3}	7.5
Phenylalanine	2.5×10^{-7}	2.5×10^{-3}	4.60
Proline	ND	ND	ND
Serine	6.7×10^{-7}	2.5×10^{-3}	8.6
Threonine	1.5×10^{-6}	2.5×10^{-3}	6.5
Tryptophan	5.8×10^{-6}	2.5×10^{-3}	4.75
Tyrosine	2.4×10^{-6}	2.5×10^{-3}	4.7
Valine	1.9×10^{-6}	$>2.5 \times 10^{-2}$	4.20

Regarding sugars, *Rhizobium* seems to be more sensitive to neutral sugars or osamines. The chemotactic behaviour of *Rhizobium* is different from that of other bacteria like *E. coli* (Mesibov and Adler 1972), *Salmonella* (Melton et al. 1978), *Bacillus* (Ordal and Gibson 1977) or *Pseudomonas* (Moench and Konetzka 1978; Moulton and Montie 1979). Chemotactic responses are weaker: the best chemotactic ratio is only 54 instead of 450 for *E. coli* with aspartate. This seems to be characteristic of *Rhizobia:* Currier and Strobel (1976, 1977a, b) also showed a chemotactic ratio of about 20. Furthermore, while most bacteria are strongly attracted to sugars (Melton et al., 1978; Ames et al. 1980) *Rhizobium meliloti* seems to have relatively weak responses to these compounds. The results are in accordance with the conclusions reached by Currier and Strobel (1976, 1977a, b) and Gitte et al. (1978). Regarding amino acidtaxis, *Rhizobium* behaviour is also quite different from that of other bacteria: generally some amino-acids are repellents for most bacteria, although not here; moreover, some of the best attractants for *Rhizobium*, like histidine or lysine, are normally weak attractants or repellents for other bacteria (Adler 1975; Melton et al. 1978; Tso and Adler 1974). Considering our results in relation to those on *Rhizobium meliloti* MVII-1 obtained by Götz et al. (1982), we note that even if chemotactic behaviour towards lysine and serine is the same for both strains, *Rhizobium meliloti* MVII-1 and Ve 26 seem to be different: aspartate is a better attractant and leucine a poorer attractant for *Rhizobium meliloti* Ve 26.

This work constituted the first stage in the study of the relation between chemotaxis and symbiosis. Preliminary results indicate that the concentration of the free amino acids present in the lucern root exudate induced and chemotactic response about $R = 23$ (Burg 1980) and especially when those exudates are from ten days old plants (an age commonly chosen for plant inoculation). Though motility (and thus chemotaxis) is not absolutely necessary for nodulation *in vitro* (direct inoculation on to the roots) it may be different in nature and chemotaxis by root exudates could play a part in the first stages of bacterial accumulation in the rhizosphere. Further investigations may provide information about the chemotaxis stage in symbiosis.

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