Attraction of *Agrobacterium tumefaciens* C58C¹ towards Sugars Involves a Highly Sensitive Chemotaxis System

By GARY J. LOAKE, ALISON M. ASHBY AND CHARLES H. SHAW*

Department of Botany, University of Durham, Science Laboratories, South Road,
Durham DH1 3LE, UK

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Motility of *Agrobacterium tumefaciens* C58C¹ consisted of long straight runs, with relatively few tumbles. Speeds of up to $60\ \mu\text{m s}^{-1}$ and runs of up to $500\ \mu\text{m}$ were recorded. The propulsive mechanism appeared to resemble that of *Rhizobium*. Chemotaxis towards carbohydrates resolved four groups of sugars: chemoattractants with peaks at $10^{-6}\ \text{M}$ (sucrose, glucose and fructose); $10^{-5}\ \text{M}$ (maltose, lactulose and galactose); $10^{-4}\ \text{M}$ (raffinose, stachyose and arabinose); and weak or non-attractants (palatinose, lactose, cellobiose and xylose). In descending order, the magnitude of the responses was as follows: sucrose $\gg$ maltose $>$ lactulose $>$ glucose $>$ galactose/fructose $>$ stachyose/arabinose/raffinose. The amino acids valine and arginine were good chemoattractants with peaks at $10^{-3}\ \text{M}$, but no significant attraction was observed with alanine, cysteine, methionine or glycine. These results are indicative of a highly sensitive chemotaxis system towards sugars in *A. tumefaciens* C58C¹, and suggest a role for this process in the ecology of the organism.

INTRODUCTION

Agrobacterium tumefaciens is a Gram-negative soil organism, frequently found in the rhizosphere (Kerr, 1969, 1974). Possession of a Ti-plasmid allows the bacterium to produce neoplastic overgrowths (crown gall) on susceptible plants. Wounding is a prerequisite for this infection, an observation explained by the fact that phenolic wound exudates, such as acetosyringone and sinapinic acid, induce operons of the Ti-plasmid virulence or *vir*-region (Okker *et al.*, 1984; Stachel *et al.*, 1985, 1986; Stachel & Zambryski, 1986; Winans *et al.*, 1986). The induced functions subsequently bring about transfer of the T-DNA from the Ti-plasmid to the plant chromosome, where its expression leads to formation of the gall (Nester *et al.*, 1984; Lichtenstein, 1986).

Acetosyringone at $< 10^{-7}\ \text{M}$, a concentration 100-fold lower than that producing maximal *vir*-induction, acts as a chemoattractant for *A. tumefaciens* (Ashby *et al.*, 1987). Only bacteria harbouring Ti-plasmids give this response, although strains cured of the Ti-plasmid respond to other compounds. We have suggested (Shaw *et al.*, 1986) that at low concentrations acetosyringone acts as a chemoattractant for Ti-plasmid harbouring strains. The bacteria thus migrate up the concentration gradient towards wounded plant cells. At higher concentrations, acetosyringone causes *vir*-induction, and thereby T-DNA transfer.

However, chemotaxis in *A. tumefaciens* is not specified by the Ti-plasmid (Ashby *et al.*, 1987) and is probably chromosomally encoded. An understanding of this system in strains cured of the Ti-plasmid is relevant to the microbial ecology and disease aetiology of *Agrobacterium*, as non-phytopathogenic forms of the organism (presumably Ti-plasmid free) predominate in soils, with virulent strains often making up less than 1% of the rhizosphere agrobacteria (Kerr, 1969). Here we report on the highly sensitive response of *A. tumefaciens* C58C¹ towards a range of sugars, many of which are abundant in plant tissues.

METHODS

Organisms and growth. All experiments were done with *A. tumefaciens* C58C¹ cured of its Ti-plasmid (Van Larebeke *et al.*, 1974) grown either in L-broth or Minimal A supplemented with glucose (MinA + G; Miller, 1972). Periodically, a fresh isolate of motile bacteria was prepared from the stock culture, by three passages through swarm plates (L-broth solidified with 0.25% agar) selecting organisms from the perimeter of the swarm at each passage. Chemotactic responses of successive isolates were reproducible. Chemotaxis medium (CM; Adler, 1973) was autoclaved and attractants filter sterilized prior to use.

Chemotaxis assay. Capillary assays (Adler, 1973) were done in duplicate, using washed exponential phase cells at a culture density of approximately 10^9 cells ml⁻¹, as previously described (Ashby *et al.*, 1987). Numbers of bacteria recovered from capillaries containing attractants were divided by control values derived from assays using chemotaxis medium alone.

Microscopy. Motility was observed at room temperature, using a Nikon Optiphot microscope with phase-contrast optics, and a Hitachi HV-720K CCTV camera. Behaviour and speed measurements were made during slow motion playback of recorded motility, using a Ferguson 3V23 video recorder and a Hitachi VM-920K video monitor.

Attractants. All sugars and amino acids were obtained from Sigma.

RESULTS

Motility

Under microscopic observation, *A. tumefaciens* C58C¹ proved to be an active swimmer. Other genotypes of *A. tumefaciens*, such as LBA4301 (Klapwijk *et al.*, 1979) or A136 (Watson *et al.*, 1975) were observed to be much less vigorously motile.

Motility was characterized by continuous straight or curved runs (Fig. 1) with few of the tumbling motions and abrupt changes of direction observed in *Escherichia coli* (Berg & Brown, 1972). Average speeds of $60 \mu\text{m s}^{-1}$ over runs of $200 \mu\text{m}$ were recorded. On longer continuous runs of over $500 \mu\text{m}$, mean speeds of $50 \mu\text{m s}^{-1}$ were maintained. Cells close to the underside of the coverslip turned consistently clockwise, while those adjacent to the surface of the glass slide moved counter-clockwise. This bias was shown by cells in rich and minimal media, and also those washed and resuspended in chemotaxis medium. Thus, *A. tumefaciens* differs from *E. coli* and more closely resembles *Rhizobium* in its motility behaviour (Götz *et al.*, 1982; Götz & Schmitt, 1987).

Attraction towards sugars

A range of monosaccharides and oligosaccharides was tested as chemoattractants for *A. tumefaciens* C58C¹. The sugars chosen were mainly abundant components of plant extracts (Kandler & Hopf, 1980). These assays showed three groups of attractants with chemotactic optima at 10^{-6} M (sucrose, glucose and fructose; Fig. 2), 10^{-5} M (maltose, lactulose and

Fig. 1. Trajectories of *A. tumefaciens* C58C¹ observed under $400\times$ magnification, using phase-contrast optics. Tracks of individual bacteria were traced onto transparent film superimposed onto a video monitor during slow motion playback of recorded motility. All runs are delineated either by the edge of the field of view or by the plane of focus, except tracks marked T, which denote runs commencing or ending in apparent tumbles. Arrowheads indicate direction of motion. (a) Cells adjacent to the underside of the coverslip; (b) cells near the glass slide surface. Track 1 was visible for 10 s during which time the cell covered $514 \mu\text{m}$. Track 2 covered $218 \mu\text{m}$ in 3.6 s. Bars represent $100 \mu\text{m}$.

Fig. 2

Fig. 3

Fig. 4

Fig. 2. Dose response curves of motility of *A. tumefaciens* towards sugars with 10^{-6} M chemotactic optima in capillary assays: ■, sucrose (α -D-glucopyranosyl- β -D-fructofuranoside); ●, glucose; ○, fructose.

Fig. 3. Dose response curves of motility of *A. tumefaciens* towards sugars with 10^{-5} M chemotactic optima in capillary assays: ●, maltose [O - α -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose]; ○, lactulose (4- O - β -D-galactopyranosyl-D-fructofuranose); ■, galactose.

Fig. 4. Dose response curves of motility of *A. tumefaciens* towards sugars with 10^{-4} M chemotactic optima in capillary assays: ○, raffinose [O - α -D-galactopyranosyl-(1 $\rightarrow$ 6)- O - α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranoside]; ●, stachyose [O - α -D-galactopyranosyl-(1 $\rightarrow$ 6)- O - α -D-galactopyranosyl-(1 $\rightarrow$ 6)- O - α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranoside]; ■, arabinose.

Fig. 5. Dose response curves of motility of *A. tumefaciens* towards amino acids in capillary assays: ●, arginine; ■, valine.

galactose; Fig. 3), 10^{-4} M (raffinose, stachyose and arabinose; Fig. 4), and a group to which no appreciable response was detected (palatinose, lactose, cellobiose and xylose; data not shown).

The most potent response was observed with sucrose as chemoattractant, the threshold being below 10^{-7} M and the peak at 10^{-6} M (Fig. 2). Glucose and fructose both produced similar curves, with a peak at 10^{-6} M. As the magnitude of the response with the monosaccharides was much lower than that of the oligosaccharide containing them, further capillary assays were done using 10^{-6} M solutions of glucose, fructose, the two monosaccharides combined, and sucrose. Glucose and fructose separately evoked similar responses, approximately 4–5 times lower than that towards sucrose. However, the mixture of the two monosaccharides produced a much higher response, of a similar magnitude to that of sucrose. This suggests that these two component monosaccharides of sucrose act synergistically in producing a chemotactic response from *A. tumefaciens* C58C¹.

The group of sugars to which little or no detectable response from *A. tumefaciens* C58C¹ was recorded includes xylose, cellobiose (4-*O*- β -D-glucopyranosyl-D-fructofuranose) and lactose (4-*O*- β -D-galactopyranosyl- α -D-glucose). Palatinose (isomaltulose; 6-*O*- α -D-glucopyranosyl-D-fructofuranose) evoked a weak response at 10^{-2} and 10^{-7} M (data not shown). These weak or non-attracting oligosaccharides are all compounded from sugars which as separate monosaccharides are good chemoattractants.

The different sugars evoked varying responses, both in terms of the molarity producing the peak response, and its magnitude. The order of response magnitudes was sucrose $\gg$ maltose > lactulose > glucose > galactose/fructose > stachyose/arabinose/raffinose. There is a trend for the least sensitive response to have the lowest magnitude.

Attraction towards amino acids

A. tumefaciens C58C¹ was tested against a restricted range of amino acids as potential chemoattractants. Valine and arginine evoked a reasonable response, with peaks at 10^{-3} M (Fig. 5) but alanine, glycine, cysteine and methionine failed to produce a significant positive response (data not shown).

DISCUSSION

Bacterial chemotaxis has been extensively studied in members of the Enterobacteriaceae, but in other bacteria is not well understood. Of the rhizosphere bacteria, only in *Rhizobium* has chemotaxis been examined in any detail (Ames *et al.*, 1980; Götz *et al.*, 1982; Ziegler *et al.*, 1986; Götz & Schmitt, 1987) although it has been observed in other bacteria prevalent in this habitat (Chet *et al.*, 1973; Bashan, 1986). Attraction of *A. tumefaciens* towards plant roots was observed by Schroth *et al.* (1971).

In many respects motility in *A. tumefaciens* C58C¹ resembles that of *Rhizobium meliloti* MVII-1 (Götz *et al.*, 1982; Götz & Schmitt, 1987) rather than *E. coli*: long straight runs, with few tumbles; run speed and distance; run curvature bias close to glass; motility not suppressed by glucose (data not shown). For *R. meliloti* MVII-1, the bias in curved trajectories is explained by unidirectional clockwise rotation of right handed helical flagella (Götz & Schmitt, 1987), a hypothesis with which our results for *A. tumefaciens* are consistent.

The similarities between *A. tumefaciens* and *Rhizobium* are less evident when analysing chemotaxis towards sugars and amino acids. Glucose and mannitol act as chemoattractants for *R. lupini* H13-3 (Götz *et al.*, 1982), and a range of carbohydrates attracts *E. coli* (Adler, 1973). However, neither organism is attracted to the same range of sugars, or with the same sensitivity, as *A. tumefaciens*, whose response to sugars is up to 1000-fold more sensitive than that of *E. coli* towards the same attractants. Chemotaxis of *A. tumefaciens* to amino acids is much less sensitive than that towards sugars, but (where positive) is of a similar sensitivity to *Rhizobium* (Götz *et al.*, 1982). However, all of the amino acids tested here are good chemoattractants for *Rhizobium* (Götz *et al.*, 1982) but alanine, cysteine, glycine and methionine do not attract *A. tumefaciens* C58C¹.

The sugars assayed were selected due to their abundance in plant extracts, or their similarity to sugars already tested. Sucrose, generally the most abundant translocated plant sugar (Kandler & Hopf, 1980) produced the most intense and sensitive response. In general, the oligosaccharides were better attractants than their component monosaccharides. This could be due to the type of receptors involved. Alternatively, it could possibly be explained by synergistic responses produced by the released monosaccharides, as suggested by our data, accompanying periplasmic degradation of the sugar. Failure of *A. tumefaciens* to metabolize other disaccharides in the periplasm may account for their behaviour as non-attractants. The effects of the tri- and tetrasaccharide may be influenced by the permeability of the outer membrane to these carbohydrates. In *E. coli*, raffinose does permeate through (Nikaido & Vaara, 1985), but nothing is known of the permeability of the outer membrane in *Agrobacterium*.

We have previously published evidence indicating that chemotaxis towards phenolic wound exudates plays a role in attracting virulent *A. tumefaciens* towards susceptible plant cells (Ashby *et al.*, 1987). The responses to amino acids and certain phenolics are similar in sensitivity to those of *E. coli* and *Rhizobium*. However, the responses evoked by *vir*-inducing phenolics and sugars are indicative of a highly sensitive chemotaxis system in *A. tumefaciens* C58C¹. As many of these carbohydrates are characteristic of plant extracts, this suggests that chemotaxis towards sugars is involved in attracting *Agrobacterium* to the vicinity of plants, which may in part explain its abundance in the rhizosphere (Kerr, 1969, 1974).

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