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Chemotaxis of *Rhizobium meliloti* to the Plant Flavone Luteolin Requires Functional Nodulation Genes†

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Luteolin is a phenolic compound from plants that acts as a potent and specific inducer of *nodABC* gene expression in *Rhizobium meliloti*. We have found that *R. meliloti* RCR2011 exhibits positive chemotaxis towards luteolin. A maximum chemotactic response was observed at 10^{-8} M. Two closely related flavonoids, naringenin and apigenin, were not chemoattractants. The presence of naringenin but not apigenin abolished chemotaxis of *R. meliloti* towards luteolin. A large deletion in the *nif-nod* region of the symbiotic megaplasmid eliminated all chemotactic response to luteolin but did not affect general chemotaxis, as indicated by swarm size on semisoft agar plates and chemotaxis towards proline in capillary tubes. Transposon Tn*5* mutations in *nodD*, *nodA*, or *nodC* selectively abolished the chemotactic response of *R. meliloti* to luteolin. *Agrobacterium tumefaciens* GMI9050, a derivative of the C58 wild type lacking a Ti plasmid, responded chemotactically to 10^{-8} M luteolin. The introduction of a 290-kilobase *nif-nod*-containing sequence of DNA from *R. meliloti* into *A. tumefaciens* GMI9050 enabled the recipient to respond to luteolin at concentrations peaking at 10^{-6} M as well as at concentrations peaking at 10^{-8} M. The response of *A. tumefaciens* GMI9050 to luteolin was also abolished by the presence of naringenin.

Symbiotic interactions between rhizobia and legume host plants result in the formation of nodular structures on the root in which the bacteria fix atmospheric nitrogen in exchange for fixed carbon. The establishment of this symbiotic association is a complex process that is still not well understood. Previous studies have identified a number of genes in the bacterial partner that are involved in nodule initiation and development. In various *Rhizobium* species, many of these nodulation genes reside in clusters on a very large plasmid. One group of nodulation genes, *nodDABC*, is common to all rhizobia studied so far (20). These common *nod* genes are required for the induction of cortical cell division and root hair curling in the host by the bacteria during the initial stages of the infection process (8, 10, 17, 18, 28). Expression of the *nodABC* genes is markedly enhanced by exposure of the bacteria to root exudates of host plants. Enhanced expression of the *nodABC* genes appears to depend on the presence of a functional *nodD* gene (16, 21, 29). Several active root exudate components have been identified and appear to be flavonoids and isoflavonoids (9, 11, 19, 25, 26, 35). Phenolic compounds that are structurally related to the inducing substances can block this stimulation of the common *nod* genes (9, 11; K. N. Peters and S. R. Long, Plant Physiol., in press). There is considerable current interest in the possibility that host specificity may be determined in large measure by the complex mixtures of *nod* gene inducers and blockers present in root exudates of potential hosts. In this regard, it has been shown that the specificity of a response to different inducing compounds correlates with the source of the *Rhizobium nodD* gene (15, 31).

The expression of virulence genes in the plant pathogenic bacterium *Agrobacterium tumefaciens* is similarly stimulated by acetosyringone and other phenolic compounds from host plants (32). Both *Rhizobium* and *Bradyrhizobium* species respond to diverse phenolic compounds by positive chemotaxis (24). Recent studies indicated that certain phenolic inducing substances also serve as chemoattractants for *A. tumefaciens* (3, 23). Such results raise the question of whether rhizobia also respond chemotactically to the phenolic substances that stimulate or block the expression of the common *nod* genes. In this paper, we describe the chemotactic responses of *Rhizobium meliloti* to flavonoids that specifically induce or block the induction of *nod* gene expression. We also address the question of what genetic elements might be required for the expression of chemotactic responses to these compounds.

MATERIALS AND METHODS

Bacterial strains and maintenance of bacteria. The strains of bacteria used in this study are listed in Table 1. *R. meliloti* RCR2011 was originally designated SU47 and is the streptomycin-sensitive parent of strain 1021. *R. meliloti* JT402 is a mutant derivative of strain 1021 that carries a transposon Tn*5* insertion located downstream of *nodE* and that is apparently neutral with respect to growth on laboratory media and nodulation (S. Long, personal communication). Unless otherwise specified, stock cultures of rhizobia and agrobacteria were maintained and grown on yeast extract-mannitol-gluconate (YEMG) (pH 7.0) containing, in grams per liter: mannitol, 5; sodium gluconate, 5; yeast extract, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.16; and NaCl, 0.1. Bacteria were also grown on either tryptone-yeast extract (TY) (containing, in grams per liter: tryptone [Difco Laboratories, Detroit, Mich.], 6; yeast extract, 3; and $CaCl_2 \cdot 2H_2O$, 0.1) or modified Götz (12) minimal medium [containing, in grams per liter: mannitol, 10; $(NH_4)_2SO_4$, 0.13; K_2HPO_4, 1.06; KH_2PO_4, 0.53; $MgSO_4 \cdot 7H_2O$, 0.25; $CaCl_2$, 0.011; NaCl, 0.006; Na_2MoO_4, 0.002; and $FeSO_4$, 0.00015]. The latter was also supplemented with 20 μg each of riboflavin, pyridoxine hydrochloride, biotin, and thiamine hydrochloride per liter.

Chemotaxis assays. Starter cultures of bacteria were grown to the stationary phase (optical density at 500 nm, 1.0) in liquid medium and then subcultured after 100-fold dilution in

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TABLE 1. Bacterial strains and relevant characteristics

Strain	Characteristics	Source (reference)
R. meliloti		
RCR2011	Wild type, Sm^s parent of 1021	J. Dénarié (27)
GMI766	Δ(*nod fixA*)*766* Spc^r	J. Dénarié (33)
GMI5382	*nodA*::Tn*5*-2208 Sm^r Nm^r	J. Dénarié (8)
GMI5387	*nodC*::Tn*5*-2217 Sm^r Nm^r	J. Dénarié (8)
TJ9B7	*nodD*::Tn*5* in strain 1021, Sm^r Nm^r	T. Jacobs (17)
JT402	402::Tn*5* in strain 1021, Sm^r Nm^r	S. Long
A. tumefaciens		
GMI9050	Spontaneous Rif^r Sm^r derivative of C58C1 (= C58 cured of the Ti plasmid)	J. Dénarié (33)
GMI9050(pGMI42)	RP4-prime derivative carrying a 290-kilobase insert of pSym2011 with Tn*5* in the *nif* region, Tc^r Ap^r Nm^r	J. Dénarié (34)

50 ml of fresh medium on a rotary shaker at 120 rpm and 30°C. Bacteria from late-exponential-phase cultures (optical density at 500 nm, 0.3 to 0.4) were harvested at a low speed (1,000 × *g*, 20 min) at room temperature in a Microfuge and then carefully suspended in chemotaxis buffer (10 mM potassium phosphate, 0.1 mM sodium EDTA [pH 7.0]) to produce a final concentration of ca. 5×10^7 bacteria per ml.

Motility and chemotaxis were assayed by using capillary tubes (1) in the chemotaxis chambers of Palleroni (22). These chambers consist of two 0.18-ml cylindrical wells joined by a narrow channel milled in a Lucite block. The channel supports a capillary tube so that its ends are centered in the wells. Sets of four 1-μl capillary tubes (Microcaps; Drummond Scientific, Broomall, Pa.) were filled with either chemotaxis buffer or a solution of the test substance at a given concentration. The wells and connecting channel of each chamber were filled with the bacterial suspension, and the capillary tubes were placed carefully with forceps in the channel. The assay plates were incubated horizontally without disturbance for 60 min at 30°C. The capillary tubes were then carefully lifted from the channel with forceps, their exteriors were washed with a thin stream of sterile distilled water (22), and their contents were pipetted into 500 μl of chemotaxis buffer. The number of bacteria that entered the capillary tubes was determined by plating onto YEMG agar plates with a model DU plating instrument (Spiral Systems, Inc., Cincinnati, Ohio). Plates were incubated at 30°C for 3 days, and colonies were counted. The temperature and pH conditions used were those determined to be optimal for *R. meliloti* (5, 12). The number of bacteria that entered a capillary tube during 60 min of incubation was calculated as an average based on four replicates for each treatment in an experiment. Each experiment was repeated at least twice. To normalize between treatments and experiments, we expressed results from chemotaxis assays in terms of the chemotaxis ratio, i.e., the number of bacteria that entered capillary tubes containing a test substance divided by the number of bacteria that entered capillary tubes containing only chemotaxis buffer. This normalization procedure has been extensively used in other chemotaxis studies but obscures variations in the absolute number of bacteria entering buffer-filled capillary tube controls in comparisons of different treatments or bacterial isolates. Such variations were rare in our experiments and are specifically noted in the text.

Significance of chemotaxis ratios. The chemotaxis ratios of the response to luteolin reported here were quite low when compared, for example, with the commonly reported ratios of the response to amino acids such as proline (5, 12), raising the question of just how reliable the luteolin values were. While the reproducibility of chemotaxis ratios was good, considerable variation was sometimes observed between counts for individual capillary tubes. In perhaps one of three treatments in an experiment, one of the four replicate capillary tubes would contain 3 to 10 times as many bacteria as the other three tubes. Similar variation was found in other studies (12, 24). Because of such variation and the relatively low ratios of the response to luteolin, we estimated the minimum chemotaxis ratio that could be considered statistically significant. The standard deviation for the number of bacteria entering buffer-filled tubes in six independent control experiments with strain RCR2011 was found to be 0.23, based on a mean normalized to 1.00. Similar standard deviations in motility behavior were found for the other strains tested. From these results we calculated that there is only a 1% probability of random deviations in chemotaxis ratios as high as 0.69. Thus, chemotaxis ratios higher than 1.69 can be regarded as biologically relevant and statistically significant at the 0.01 level.

Chemotactic migration through agar. Swarm plates containing semisoft (0.3%) YEMG agar was inoculated in the center with the strain to be tested and incubated at 30°C for 5 days. The diameter of swarm colonies was found to increase linearly with time, so the rate of swarm growth was determined for each strain after linear regression analysis.

Chemicals. The following chemicals were tested as chemoattractants: 3′,4′,5,7-tetrahydroxyflavone (luteolin), 4′,5,7-trihydroxyflavanone (naringenin), and 4′,5,7-trihydroxyflavone (apigenin). Luteolin was obtained from Carl Roth GmbH Co., Karlsruhe, Federal Republic of Germany, and naringenin and apigenin were obtained from Sigma Chemical Co., St. Louis, Mo. Stock solutions of these compounds were divided into aliquots and maintained for up to 3 weeks in methanol at −20°C.

RESULTS

The kinetics of entry of *R. meliloti* cells into buffer-filled capillary tubes was examined and found to be roughly linear for at least 90 min (data not shown). In six independent experiments, the average number of *R. meliloti* RCR2011 cells which entered buffer-filled capillary tubes during the first 60 min was found to be 2.5×10^3. This corresponds to about 0.01% of the total population of bacteria initially added to the chamber. The concentration of bacteria in the control tubes after 60 min was approximately 5% of that in the wells. Incubation for an additional 60 min increased that percentage to about 7%. Examination of the bacteria by phase-contrast microscopy showed that a sizable fraction of the cells displayed normal motility, with typical runs and stops, even after centrifugation and suspension in chemotaxis buffer.

TABLE 2. Motility and general chemotaxis of bacterial isolates

Strain	Mutation	No. of bacteria/ tube (10^3)[a]	Migration rate (mm/h)[b]
R. meliloti			
RCR2011	None (wild type)	2.5 ± 0.5	0.48a
GMI766	Deletion	2.5 ± 0.3	0.39a
GMI5382	*nodA*	1.5 ± 0.6	0.45a
GMI5387	*nodC*	2.3 ± 0.4	0.43a
TJ9B7	*nodD*	2.8 ± 0.3	0.45a
JT402	Neutral	0.7 ± 0.1	0.10b
A. tumefaciens			
GMI9050		1.7 ± 0.1	1.03c
GMI9050(pGMI42)		1.4 ± 0.1	0.97c

[a] Motility was assessed by determining the average number of cells which entered buffer-filled capillary tubes during a 1-h period. Values are presented with 95% confidence intervals and represent averages from four replicates per experiment and two to nine independent experiments.

[b] General chemotaxis was determined by measuring the rate of increase in swarm colony diameter on semisoft YEMG agar. Values followed by the same letter are not significantly different ($P = 0.05$).

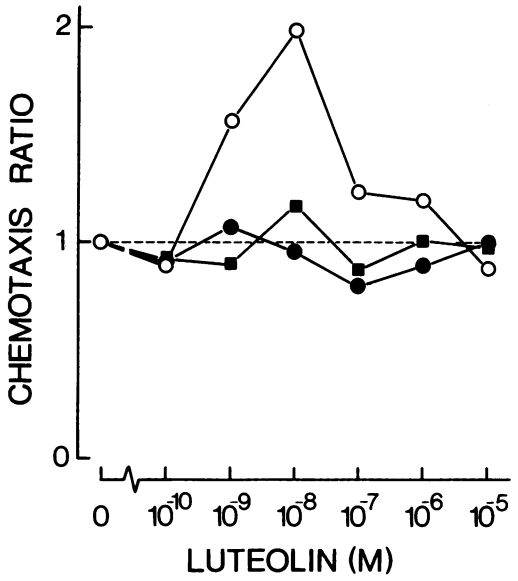

FIG. 1. Chemotactic responses of an *R. meliloti* wild-type isolate, *nif-nod* deletion mutant, and *nodD*::Tn*5* mutant to different concentrations of luteolin. Assays were performed as described in Materials and Methods. Average background levels of bacteria in capillary tubes filled with chemotaxis buffer are given in Table 2. Symbols: ○, wild-type *R. meliloti* RCR2011; ●, deletion mutant GMI766; ■, *nodD* mutant TJ9B7. Results are averages from six, three, and two experiments for RCR2011, GMI766, and TJ9B7, respectively, with four replicates per treatment in each experiment. The concentration of luteolin was determined by absorbance measurements prior to dilution by using an extinction coefficient of $10^{4.2}$ at 256 nm.

In control experiments, the accumulation of bacteria in capillary tubes filled with buffer was found to be comparable for all the isolates tested, with a range of 1,400 to 2,800 bacteria per capillary tube over a 60-min period for *R. meliloti* and *A. tumefaciens* (Table 2). The only exception was *R. meliloti* JT402, for which the average accumulation was about 700 bacteria per capillary tube. This isolate is a mutant of strain 1021, which is a streptomycin-resistant derivative of the wild-type strain RCR2011. In further tests, the general chemotaxis behavior of these different isolates was examined by comparing the relative spread of their colonies on semisoft agar swarm plates. Wild-type *R. meliloti* RCR2011 and each of the mutant derivatives had comparable migration rates, with the exception of JT402 (Table 2). Swarm colonies of *A. tumefaciens* increased in size about twice as rapidly as did *R. meliloti* swarm colonies.

The wild-type isolate *R. meliloti* RCR2011 was attracted by very low concentrations of luteolin (Fig. 1). Although the chemotaxis ratios were relatively low, both the magnitude of the response to luteolin and the concentration eliciting a maximal response proved to be readily reproducible between experiments. Under the present assay conditions, luteolin was most effective as a chemoattractant at a concentration of 10^{-8} M, with a diminished response at either 10^{-9} or 10^{-7} M. The maximum chemotaxis ratio obtained with RCR2011 in response to luteolin was approximately 2. Similar chemotactic responses were observed with RCR2011 cells which had been cultured on modified Götz minimal growth medium or on a very rich growth medium (TY). Neither naringenin nor apigenin elicited a statistically significant chemotactic response at concentrations between 10^{-10} and 10^{-5} M (chemotaxis ratios ranged from 0.77 to 1.21).

To test whether luteolin acts as a specific attractant for the bacteria or acts more generally to increase the overall level of motility without affecting the bias of flagellar motor switching, we added luteolin to both capillary tubes and the suspension of bacteria outside the capillary tubes. The presence of 10^{-8} M luteolin in the suspension outside the capillary tubes reduced the movement of RCR2011 into the tubes to background levels (chemotaxis ratio, 0.94). The entry of the bacteria into buffer-filled capillary tubes was reduced by 37%. Thus, the presence of luteolin does not appear to enhance general motility. The accumulation of bacteria inside the tubes appears to require a concentration gradient of luteolin.

The effects of naringenin and apigenin on the chemotactic response of *R. meliloti* to luteolin was also examined. The presence of naringenin in the capillary tubes at a concentration of 10^{-7} M was found to block the chemotaxis of wild-type cells towards luteolin at concentrations between 10^{-9} and 10^{-7} M (Table 3). The addition of apigenin had no appreciable effect.

R. meliloti GMI766, a derivative of RCR2011 with a large deletion in the *nif-nod* region of the symbiotic megaplasmid, did not respond chemotactically to luteolin at any of the concentrations tested (Fig. 1). This deletion mutant formed spreading colonies on swarm plates very similar in size to those formed by the parent (Table 2) and responded normally to proline in capillary tube assays (data not shown), indicating that general chemotaxis and motility behavior

TABLE 3. Chemotaxis of *R. meliloti* RCR2011 towards luteolin in the presence of naringenin or apigenin[a]

Luteolin concn (M)	Chemotaxis ratio in the presence of:		
	Buffer	10^{-7} M naringenin	10^{-7} M apigenin
0	1.00	1.07	1.00
10^{-9}	2.17	1.33	1.88
10^{-8}	2.41	1.00	2.05
10^{-7}	0.94	1.04	1.78

[a] The background level in these experiments was 1,800 ± 300 bacteria per capillary tube.

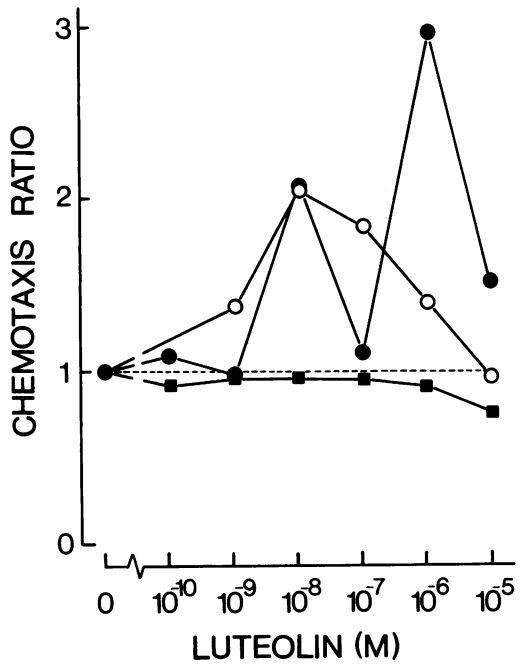

FIG. 2. Chemotactic responses of *A. tumefaciens* derivatives to different concentrations of luteolin and the effect of naringenin. Assays were performed as described in Materials and Methods. Average background levels in buffer-filled capillary tubes are given in Table 2. Symbols: ○, *A. tumefaciens* GMI9050; ●, *A. tumefaciens* GMI9050(pGMI42); ■, *A. tumefaciens* GMI9050(pGMI42) in the presence of naringenin. Naringenin was added to the capillary tubes at a concentration 10-fold higher than that of luteolin. Results are averages from two experiments with four replicates per treatment in each experiment.

were not appreciably affected by this deletion. Insertion of Tn*5* into the *nodD* gene was found to abolish the chemotaxis of *R. meliloti* towards luteolin (Fig. 1). Tn*5* insertions into *nodA* (strain GMI5382) and *nodC* (strain GMI5387) also abolished the chemotactic response of RCR2011 to luteolin (chemotaxis ratios ranged from 0.73 to 1.14 at concentrations between 10^{-10} and 10^{-5} M). The insertion of Tn*5* into a "neutral" location in mutant JT402 had no discernable effect on its dose-response behavior in comparison with that of RCR2011 (data not shown), indicating that Tn*5* per se does not abolish the specific chemotactic response. The *nodA*, *nodC*, and *nodD* mutant derivatives all formed spreading colonies on swarm plates in a manner comparable to that of the parent (Table 2).

A. tumefaciens GMI9050 was found to respond chemotactically to luteolin in a manner similar to that of *R. meliloti* RCR2011, with a maximum response at 10^{-8} M and a maximum chemotaxis ratio of about 2 (Fig. 2). The transfer of a 290-kilobase *nif-nod*-containing sequence of the symbiotic megaplasmid from *R. meliloti* RCR2011 to *A. tumefaciens* GMI9050 had no major effect on its response to 10^{-8} M luteolin but did create a new peak of chemotactic response to luteolin at a concentration of 10^{-6} M (Fig. 2). Bacteria recovered from the capillary tubes in these experiments were uniformly resistant to kanamycin, indicating that the segment of DNA from *R. meliloti* was still present. The addition of naringenin at concentrations 10-fold higher than those of luteolin abolished the responses of GMI9050 (pGMI42) at both 10^{-6} and 10^{-8} M luteolin (Fig. 2).

DISCUSSION

Chemotaxis and motility have been found to make a number of important contributions to the symbiotic interactions of rhizobia with their hosts, including effective movement through the soil (30), contact and adherance to the host root (5a), inhibition of attachment of other rhizobia to the root surface (5a), formation of highly localized bacterial "clouds" or swarms on the infectible surface of the root (13), efficient nodule initiation (5a), rapid infection development (5a), and competition for nodule occupancy (2, 5a, 13). A good deal remains to be learned about how chemotaxis and motility contribute to these phenomena. Identification of the host substances that serve as chemoattractants for rhizobia, especially under natural field conditions, is one important concern. A number of sugars and amino acids commonly present in root exudates can serve as chemoattractants for rhizobia (e.g., see references 5 and 12), although the concentrations of these readily metabolized compounds might be quite low in a well-populated rhizosphere. There have also been reports that rhizobia are selectively attracted to certain proteins present in host root exudates (6, 7). It now appears that certain phenolic compounds in host root exudates can serve as potent and specific chemoattractants. Our studies indicate that the ability of these phenolic compounds to function as chemoattractants appears to be closely related to their ability to stimulate the expression of the common nodulation genes in the bacteria. The parallel effects of specific phenolic compounds on chemotaxis and induction of *vir* gene expression in *A. tumefaciens* (23) suggest that this pattern of coordinated responses may be of general importance to plant-microbe interactions.

In the present work, we found that both *R. meliloti* and *A. tumefaciens* were attracted to low concentrations of luteolin. The concentration of luteolin which elicited a maximal chemotactic response in *R. meliloti* was approximately 10-fold lower than that required for full induction of the common *nod* genes in *R. meliloti* (25). It appears that *R. meliloti* should be able to detect luteolin coming from the host root at concentrations between 10^{-10} and 10^{-9} M. As a consequence, motile cells in the rhizosphere population should move chemotactically towards the host until the luteolin concentration reaches about 10^{-7} M. At that concentration, chemotaxis towards luteolin is diminished (Fig. 1) and the induction of *nod* gene expression is rapid (25).

Chemotaxis of *R. meliloti* cells towards luteolin appears to be chemically specific. Naringenin and apigenin, which are close structural relatives of luteolin, did not elicit a detectable chemotactic response under the present assay conditions. Since *R. meliloti* and *A. tumefaciens* responded to luteolin without prior incubation or growth of the cells in the presence of this substance, it appears that chemotaxis to luteolin is a constitutive trait, at least at the low levels seen here. It remains to be seen whether a greater responsiveness to luteolin can be induced in these bacteria or whether chemotactic responses to naringenin and apigenin are inducible.

It is of interest that naringenin effectively blocked the chemotactic response of *R. meliloti* cells to luteolin (Table 3), just as it blocks the enhancement of *nodABC* gene expression by luteolin (Peters and Long, in press). The presence of apigenin, on the other hand, had some, but no major, effects on chemotaxis towards luteolin and on the enhancement of *nodABC* gene expression by luteolin. Since luteolin, naringenin, and apigenin have very parallel effects on these two phenomena, the response pathways for the

induction of *nod* genes and specific chemotaxis may share one or more common elements.

Although the *nif-nod* deletion mutant GMI766 lost its ability to respond chemotactically to luteolin (Fig. 1), it appeared to be normal in its colony formation on semisoft agar swarm plates and in its response to proline, suggesting that the genes responsible for general motility and chemotaxis are not located in the *nif-nod* region of the symbiotic megaplasmid, in agreement with a recent report in which various *che*, *mot*, and *fla* genes were mapped to a cluster on the *R. meliloti* chromosome (36).

Inactivation of *nodD* eliminated both luteolin enhancement of *nodABC* gene expression (21) and chemotactic responses to luteolin (Fig. 1), again indicating that these two response pathways may share a common element. The exact role of *nodD* in chemotaxis towards luteolin and in the induced expression of *nodABC* remains to be established. *nodD* seems to be expressed constitutively (21). It is not clear whether the *nodD* product interacts directly with luteolin or whether its synthesis or activity is indirectly responsive to luteolin.

The loss of the chemotactic response to luteolin upon inactivation of *nodA* and *nodC* was unexpected. The effects of a Tn*5* insertion in *nodA* might be explained by its effect on the downstream expression of *nodB* or *nodC*. Since the end of the *nodABC* operon has yet to be clearly established, it is also possible that the *nodA* and *nodC* mutations disrupt chemotaxis towards luteolin by preventing the expression of one or more unidentified genes downstream in the same operon. Nonetheless, it seems clear that this operon simultaneously governs three distinct phenotypes: chemotactic sensitivity to luteolin; the induction of root hair curling; and the induction of cortical cell division in the host. There are inadequate data available to propose a model that could account for this diversity of roles. We are presently attempting to identify the gene(s) required for a chemotactic response to luteolin and are trying to determine the role of nodulation genes in this response.

Luteolin occurs in a variety of dicotyledonous plant species (14), so it is perhaps not surprising that it would serve as a potent chemoattractant for a pathogen of dicots, *A. tumefaciens* (Fig. 2). A recent report has indicated that *A. tumefaciens* chemotactically responds to and/or metabolizes a variety of monocyclic phenolic substances that act as inducers of virulence genes (23). Chemotaxis towards these substances is a constitutive trait and does not require the presence of the Ti plasmid (23). Another recent report has provided evidence that acetosyringone, another monocyclic phenolic inducer of *vir* gene expression (32), can act as a potent chemoattractant for *A. tumefaciens* (3). Acetosyringone is maximally effective as a chemoattractant at concentrations of about 10^{-7} M, with chemotaxis ratios of 3 to 4. Chemotaxis towards acetosyringone was found to depend on the presence of the Ti plasmid (3), just as chemotaxis towards luteolin in *R. meliloti* was dependent on the *nif-nod* region of the symbiotic megaplasmid (Fig. 1). However, the chemotactic response of *A. tumefaciens* to other monocyclic phenols (23) and to luteolin (Fig. 2) were not dependent on the Ti plasmid, so chemotactic sensitivity to *vir* inducers need not be clustered with the *vir* genes.

It is of interest that transfer of the *nif-nod* region of the *R. meliloti* symbiotic megaplasmid to *A. tumefaciens* could establish a second chemotactic response to luteolin (Fig. 2). *R. meliloti* and *A. tumefaciens* are closely related members of the *Rhizobiaceae*, so they may have homologous elements in their chemotactic response pathways that allow interchangeable expression of chemotactic sensitivity. The fact that naringenin was able to block the ability of both *A. tumefaciens* and *R. meliloti* to respond chemotactically to luteolin is consistent with this idea.

Bergman et al. (4) recently observed that mutations in *R. meliloti* which abolished chemotactic responses to ordinary sugars and amino acids did not abolish the formation of localized swarms of motile cells at discrete sites on the root surface. Based on these results, Bergman et al. suggested that *R. meliloti* may have a dual pathway for chemotactic responses, with one branch of the pathway involved in responses to ordinary nutrients and the other branch involved in responses to specific host plant signal substances. The results obtained in our studies of chemotaxis towards various flavonoids in *R. meliloti* provide further support for this idea, since mutations in the *nif-nod* region appear to have no appreciable effects on general chemotaxis, as measured by colony spreading on swarm plates.

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