# Motility of *Pseudomonas syringae* pv. *glycinea* and its Role in Infection

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## ABSTRACT

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Flagellar motility in *Pseudomonas syringae* pv. *glycinea*, as determined by capillary assay, is optimal at growth temperatures of 17–27 C and inhibited by higher temperatures. Motility is optimal at pH 6–7 and in the presence of $10^{-5}$ M ethylenediamine tetraacetic acid, but reduced motility occurs in the absence of and in the presence of higher concentrations of EDTA. Motility is stimulated by exogenous energy sources such as glycerol, sodium citrate, and asparagine in the presence of oxygen. *P. s. glycinea* is attracted to leaf extracts from both susceptible and resistant soybean cultivars. A nonmotile strain (Nm7), obtained by mutagenesis with ethyl methanesulfonate, and its motile revertant (Mr7) were as pathogenic as the wild-type strain on susceptible soybean (Wells II) leaves. Externally applied inoculum (leaf dip) of Mr7 resulted in significantly more lesions on susceptible soybean leaves (water-soaked by vacuum infiltration) than did Nm7 at a concentration of $4 \times 10^5$ colony-forming units per milliliter.

*Additional keywords*: chemotaxis, soybean bacterial blight.

Most plant-pathogenic bacteria demonstrate flagellar motility (19). Motility and chemotaxis are beneficial characteristics, because they appear to give cells a survival advantage by allowing them to actively compete for favorable environments while avoiding conditions that are deleterious (13,16,18,26,33). In certain bacterial species, motility increases the infection potential of cells by allowing greater access to infection courts (4,26). Some plant-pathogenic bacteria are chemotactically attracted to host plant extracts (7,10,11,29), suggesting that chemotaxis also may play a role in the location of infection sites. Studies with *Pseudomonas syringae* pv. *phaseolicola* have shown that chemotaxis does play such a role in bean leaf invasion (22). *P. lachrymans* was attracted to extracts from susceptible and resistant cucumber plants (7), whereas *Xanthomonas oryzae* was attracted to exudates from susceptible rice plants more strongly than to exudates from resistant rice plants (11).

Environmental conditions such as pH, temperature, chelating agents, oxygen, and exogenous energy sources affect bacterial motility under laboratory conditions (2,30). Most research on these factors has focused on commonly studied bacteria, such as *Escherichia coli*, rather than on plant-pathogenic bacteria. Raymundo and Ries (30) were the first to describe these factors for a bacterial plant pathogen, *Erwinia amylovora*.

We describe the factors affecting motility for a polarly flagellated bacterium, *P. s. glycinea* (Coerper) Young, Dye, and Wilkie, which causes bacterial blight of soybean. Also reported is the attraction of this organism to leaf extracts from both susceptible and resistant soybean (*Glycine max* (L.) Merr.) cultivars and the greater infection potential of motile cells over nonmotile cells, as has been observed in *P. phaseolicola* (26). Preliminary results have been published (15).

## MATERIALS AND METHODS

**Media and bacterial strains.** Bacteria were grown on King's medium B (KB) (31); a modified version of King's B in solid (MKB), liquid (MKBB, modified King's B broth), and semisolid (SMKB) forms; Vogel-Bonner minimal medium (VB) (34); and L broth without dextrose (6). Modified King's medium B contains casein hydrolysate instead of proteose peptone No. 3, and the solid and semisolid forms contain agar at 15 and 3.5 g/L, respectively. All growth media were adjusted to pH 7 with KOH or HCl and were sterilized for 15 min at 121 C.

An isolate of *P. s. glycinea* (Pg-31) was obtained from S. M. Lim of this department. The production of typical water-soaked lesions on leaves of soybean (cultivar Wells II) and a hypersensitive reaction (HR) on leaves of tobacco (*Nicotiana tabacum* L. 'Samsun' NN) was checked periodically with this isolate and the mutants described below. Wells II soybean and Samsun NN tobacco were used throughout this research, unless otherwise indicated.

Motile cells were selected from the original isolate by stab inoculating into the center of SMKB plates and reisolating from the edge of the swarm after 48 hr of incubation at room temperature. This procedure was repeated several times. The final isolate (wild-type) and the mutants described below were stored in MKBB at −80 C. All cultures were revived from cold storage before each experiment to avoid repeated subculturing.

**Selection of mutants.** This procedure is a modification of a technique used by Ordal and Adler (24). Wild-type cells were grown for 24 hr in MKBB. A portion of this culture was transferred to L broth and incubated 3 hr to a final concentration of approximately $10^7$ cfu/ml ($A_{590nm}$ = 0.04). Cells in 5 ml of this suspension were then washed twice by centrifugation in a buffer containing 0.1 M potassium phosphate, 0.2 mM ethylenediamine tetraacetic acid (EDTA), and 2 mM potassium glutamate at 1,900 *g* for 4 min at room temperature. The cells were resuspended in 5 ml of buffer with 3% ethyl methanesulfonate (v/v). After 80 min at room temperature, the cells were washed twice in 5 ml of L broth, resuspended in 1 ml of L broth containing 8% dimethyl sulfoxide, incubated with shaking at room temperature for 10 min, and stored at −80 C.

To select nonmotile mutants, mutagenized cells were grown in 15 ml of MKBB at 25 C on a shaker for 24 hr. The culture was diluted 1:100 in fresh broth (MKBB) and 0.01 ml was mixed in a plate with 10 ml of soft agar (SMKB) at 45–50 C. After 24–48 hr at 24 C, colonies of nonmotile cells were observed suspended in a "hazy" background of motile cells. Several of these colonies were carefully transferred to hard agar (MKB) plates and incubated 48

hr. MKB was used because KB resulted in a more filamentous cell type that appeared on microscopic examination to swim more slowly than the shorter cells grown in MKB. Isolated colonies were checked microscopically for motility. Nonmotile cells were subcultured on MKB, transferred to MKBB, shaken overnight, and stored at −80 C. Five nonmotile isolates, including Nm1, Nm6, Nm7, Nm9, and Nm15, were obtained in this manner from separate colonies on the original soft agar plate.

Motile revertants were obtained by inoculating the center of SMKB plates with an overnight broth culture of each nonmotile isolate. After several days, revertants could be identified by the appearance of "flares" of bacteria spreading away from the inoculation point. Cells were isolated from the outer edge of flares and transferred to fresh SMKB. This was repeated several times, after which isolates were checked microscopically for motility, transferred to MKBB, incubated with shaking at 25 C for 12 hr, and stored at −80 C. Ten motile revertant isolates, Mr1A, Mr1B, Mr6A, Mr6B, Mr6C, Mr7A, Mr7B, Mr7C, Mr9, and Mr15, were obtained.

All isolates were compared to the wild-type for pathogenicity, spreading ability on soft agar, and growth on minimal medium. Isolates Mr7 and Nm7 were most similar to the wild-type and were chosen for further tests, including comparison of motility and chemotaxis (capillary assay) and generation time. Not all isolates were compared, due to time and labor constraints. To compare pathogenicity of isolates, inoculum was prepared by streaking cells from mid-log phase MKB broth cultures onto KB agar plates. Cells were gently washed from the agar surface after 24 hr of incubation at room temperature and suspended with sterile distilled water to a concentration of $10^7$ cells per milliliter. Leaves of 15–21-day-old soybean plants were stabbed 15–20 times with the needle of a syringe containing the bacterial suspension. The suspension was then sprayed from the syringe onto the surface of leaves, and symptoms were observed 7 days later. Pathogenicity of isolates was checked every 3 mo. To determine growth on minimal medium, mid-log phase cultures in MKBB were streaked onto VB plates and growth was observed after 24 hr.

The procedures used to determine motility and chemotaxis by capillary assay and spreading ability on soft agar are described below. For motility comparison of Mr7 and Nm7 by capillary assay, the treatments were arranged in a split-plot design with time as main plots and isolates as subplots. The experiment was repeated four times with two replications per experiment. For comparison of chemotaxis of isolates Mr7, Nm7, and wild-type to soybean leaf extract, treatments were arranged in a randomized complete block design. Three experiments were done, with four replications per experiment. Chemotaxis buffer was at pH 7 with $10^{-4}$ M EDTA. Generation times were determined by growing cells (retrieved from cold storage) in 15 ml of MKBB to mid-log phase (24 hr) and transferring 0.1 ml of cells to 15 ml of fresh MKBB for 12 hr (mid-log phase). An aliquot (0.5 ml) of this culture was transferred to 50 ml of MKBB in 500-ml sidearm flasks. Cultures were shake-incubated at 85 oscillations per minute at 26 C, and turbidity measurements were taken every 2 hr with a Klett-Summerson photoelectric colorimeter with a blue filter. Generation times were calculated at mid-log phase of growth by dilution plating on KB (28). Treatments were arranged in a completely randomized design, and three experiments were done, with two replications per experiment.

**Motility/chemotaxis assays.** One type of assay for motility and chemotaxis measures the spreading ability of isolates on soft agar. Broth (MKBB) cultures (24 hr) of motile revertant (Mr), nonmotile (Nm), and wild-type isolates at mid-log phase were stab-inoculated into SMKB plates. The experimental design was a completely randomized design with two subsamples (inoculations per plate) in each of four replications. Colony diameters were measured after 24 hr at 24 C and were averaged over subsamples and isolates.

The capillary assay is a modification of the procedure originally devised by Adler (1) and employed by Bayot (3). Cells used in the assay were retrieved from cold storage (−80 C) and incubated 24 hr in 15 ml of MKBB at 25 C in a shaker bath at 100–120 oscillations per minute. A portion of this culture was transferred to fresh broth and incubated overnight under identical conditions. After reaching a turbidity of 0.1–0.3 at 590 nm (mid-log phase), cells were harvested by centrifugation of 3–4 ml at 1,900 *g* for 8 min at 4 C. The cells were then washed twice by alternate centrifugation and resuspension in cold (4 C) motility or chemotaxis medium to a concentration of $4 \times 10^7$ cfu/ml. Cell pellets were resuspended gently to avoid flagellar breakage. Unless otherwise noted, motility medium consisted of $5 \times 10^{-2}$ M potassium phosphate buffer and $10^{-5}$ M EDTA. Chemotaxis medium consisted of motility medium plus $5 \times 10^{-3}$ M $MgCl_2$, which was added because it enhanced chemotaxis to soybean leaf extracts and sodium citrate (unpublished data). Motility and chemotaxis media were adjusted to the proper pH, filter-sterilized with a Nalge filter apparatus (0.2 $\mu$m), and stored at 4 C in sterile flasks. In all cases the motility and chemotaxis media were agitated vigorously for 10–15 sec to provide adequate oxygen before cells were added.

A sterile glass slide, U-shaped capillary, and a coverslip were used to form a small chamber into which 0.2 ml of the bacterial suspension was placed (1). All glassware was rinsed twice with double glass-distilled water and dried in an oven or autoclaved 15 min at 121 C. To measure motility, a 1-$\mu$l capillary with one end sealed and containing motility medium was placed into the bacterial suspension. The number of bacteria in the capillary at the end of the incubation period is a function of their rate of movement. Chemotaxis assays were identical to motility assays except that chemotaxis medium was used instead of motility medium and a potential attractant was dissolved in the chemotaxis medium put into the capillary.

All assays were done at 24 C for 30 min, after which the capillaries were removed and rinsed with sterile distilled water. The closed ends were then broken, the contents transferred to sterile 1% peptone water, and appropriate dilutions plated on KB plates (0.1% peptone was substituted for 1% peptone in later experiments with no change in plating efficiency). After 48 hr the resulting colonies were counted. Each treatment was replicated four times on two or more separate days (trials). The number of bacteria per capillary was based on duplicate plate counts.

**Factors affecting motility.** Effects of growth temperature, pH, chelating agent, energy source, and oxygen on motility were studied by capillary assay and/or microscopic examination (phase contrast, 400×). Cells were grown at 17, 20, 23, 27, 30, and 33 C to determine the temperature that offered the best compromise between good motility and adequate growth. Capillary assays were then done at 23 C, using cells grown at each temperature. Treatments were arranged in a completely randomized design. Four experiments were done, with four replications per experiment. The number of cells previously grown at one of the temperatures listed above that accumulated per capillary was divided by the number of cells previously grown at 23 C that accumulated per capillary to obtain relative motility values. Relative growth responses were determined turbidimetrically.

The capillary assay was used to examine the effect of pH on motility. Cells were grown in MKBB at pH 7, and the motility of cells was assayed in motility medium adjusted to pH 5, 6, 7, or 8. Treatments were arranged in a randomized complete block design. Three experiments were done, with four replications per experiment. The effect of growth medium pH on motility was determined microscopically by growing cells on KB at pH 5, 6, 7, 8, 9, or 10 and suspending portions of these cultures in motility medium (pH 7) on microscope slides. Cells were rated for vigorous motility (speed) and proportion of motile cells. The EDTA concentration in motility medium was varied from zero to $10^{-1}$ M to determine the effect of a chelating agent on motility by capillary assay. Treatments were arranged in a completely randomized design and four experiments were done, with four replications per experiment.

Glucose, sucrose, galactose, fructose, mannitol, glycerol, glutamine, asparagine, sodium citrate, and arginine were added individually to the motility medium at pH 6 with $10^{-5}$ M EDTA to determine whether the addition of an energy source would stimulate motility. The effects were determined by capillary assay in three experiments, with four replications per experiment and

with treatments arranged in a randomized complete block design.

**Preparation of soybean leaf extract.** Soybean (Wells II and Williams) leaf extracts were prepared for use as attractants of *P. s. glycinea* in chemotaxis assays. Plants were grown for 15–21 days in a greenhouse or in a growth chamber with 17 hr of light daily and a mean temperature of 26 C. Five grams of detached leaflets was placed into a vacuum flask with 100 ml of chemotaxis medium. Leaves were swirled occasionally while a vacuum (381–508 mm of Hg) was repeatedly pulled and rapidly released. After 5–10 min of this treatment, the water-soaked leaflets were removed and discarded. The extract was sterilized by vacuum filtration through a Nalge 0.2-$\mu$m filter unit and stored in sterile flasks in the refrigerator until used.

**Chemotaxis toward leaf extracts.** Chemotaxis of the wild-type isolate toward a susceptible (Wells II) and a resistant (Williams) soybean cultivar was determined by capillary assay. Susceptibility and resistance were confirmed by inoculating leaves with a suspension of $10^8$ cells per milliliter, using a syringe as described above under selection of mutants. Cells were suspended to $4\times 10^7$ cfu/ml in chemotaxis medium consisting of $5\times 10^{-2}$ M potassium phosphate buffer at pH 6.5, $10^{-5}$ M EDTA, $5\times 10^{-3}$ M $MgCl_2$, and $10^{-2}$ M glycerol. The experiment was repeated three times, with four replications per experiment. Treatments were arranged in a randomized complete block design.

**Plant inoculations.** To determine whether motile cells are more infective to soybean leaves than nonmotile cells, a leaf dip inoculation procedure was developed similar to one used by Panopoulos and Schroth (26) with *P. phaseolicola*. In this technique, leaves were not stab inoculated as they were for comparing pathogenicity of isolates (described above). Instead leaf tissue was made more accessible to bacterial invasion via vacuum infiltration with distilled water in a specially constructed vacuum chamber. This chamber allowed the inverted suspension of whole soybean plants and submersion of leaves in distilled $H_2O$. In this chamber, a vacuum of 381–508 mm of Hg could be obtained and released repeatedly. After 10 min of repeated vacuum infiltration of four plants, submerged leaves were water-soaked. Plants were removed from the chamber, and the leaves were dipped into 400 ml of inoculum of either Mr7 or Nm7 cells for 2 min (two plants for each inoculum type). Inoculum was prepared as described previously for capillary chemotaxis assays, except that the final cell density was adjusted to $4\times 10^5$ cfu/ml in chemotaxis medium. Inoculations were made on nearly fully expanded second or third trifoliate leaves (second trifoliates used in the first trial, third trifoliates used in the second trial) on soybean plants grown 15–21 days in the growth chamber. Plants were removed from the inoculum, rinsed in deionized water in a beaker, and placed in a Percival dew chamber at 20 C with 20 hr of light and 4 hr of darkness. This procedure was repeated with two additional groups of four plants, each of which were water-soaked, left in the inoculum (for 5 min and 10 min, respectively), and then placed in the dew chamber. Separate beakers of inoculum were used for each time period to avoid the possible adverse effect of residual leaf products or washings from previously inoculated plants.

Lesions were counted 6–7 days later. The treatments were arranged in a completely randomized design, and the number of lesions per leaflet on six leaflets (three leaflets per plant) were counted per treatment per experiment. The experiment was repeated twice. Leaflet areas were measured with a Li-Cor model LI-3100 area meter with 1.0 $mm^2$ resolution.

**Data analysis.** Data were analyzed by analysis of variance. When appropriate, mean comparisons were done with Fisher's least significant difference mean separation test (FLSD, $P=0.05$).

## RESULTS

**Factors affecting motility.** *P. s. glycinea* was most motile when grown at cooler temperatures. Microscopic examination of cultures revealed more vigorous cell motility at a growth temperature of 17 C than at 33 C. This was confirmed by capillary assay in which cell motility decreased steadily with increasing growth temperature (Fig. 1). Cells were more motile at lower temperatures, but their growth rates were not as high as those of cells cultured at 23–30 C (optimal range) (5). All future assays used 25 C because it was most convenient to use and offered the best compromise between producing cells with adequate motility and good growth rates. Motility was increased above control values by adding EDTA to the motility medium (Fig. 2). Maximum motility was observed at $10^{-5}$ M EDTA. Higher concentrations resulted in a sharp decline in motility below the control, with essentially no cells motile at $10^{-1}$ M. Therefore, motility and chemotaxis media used in all future experiments contained $10^{-5}$ M EDTA.

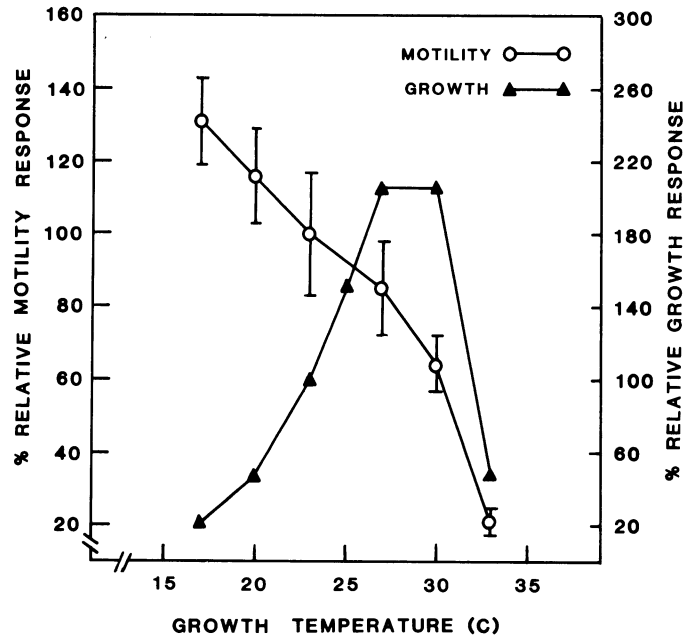


**Fig. 1.** Effect of incubation temperature on motility and on growth of *Pseudomonas syringae* pv. *glycinea*. Responses are adjusted to 100% relative to actual values at 23 C. Actual values for motility are colony-forming units per capillary and for growth $A_{590nm}$. Motility assays were run for 30 min with $4\times 10^7$ cells per milliliter suspended in $5\times 10^{-2}$ M potassium phosphate buffer and $10^{-5}$ M ethylenediamine tetraacetic acid at pH 6. Bars represent standard error of the mean. Bars in growth curve are hidden by symbols.

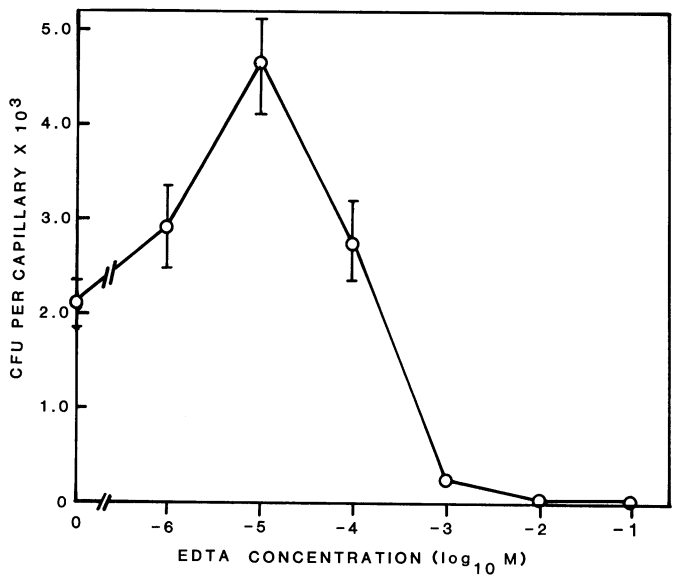


**Fig. 2.** Effect of concentration of ethylenediamine tetraacetic acid (EDTA) on motility of *Pseudomonas syringae* pv. *glycinea* as determined by capillary assay. Assays were run for 30 min with $4\times 10^7$ cells per milliliter suspended in $5\times 10^{-2}$ M potassium phosphate buffer (pH 6) without glycerol. Bars represent standard error of the mean.

The optimum pH for motility, as determined by capillary assay with motility medium adjusted to various pH levels, was 6–7 (Fig. 3). These cells had been grown in MKBB at pH 7. Cells grown on MKB agar at various pH levels were suspended in motility medium at pH 7 and examined microscopically for motility. Cells grown at pH 6 and 7 possessed the highest percentage of and the most vigorous motile cells. A small percentage of cells produced at other pH values (5, 8, 9, and 10) were motile but less vigorous.

Asparagine ($10^{-3}$ M), glycerol ($10^{-3}$ M), and sodium citrate ($10^{-2}$ M) resulted in accumulation of 13,806, 8,250, and 9,670 cfu per capillary, respectively, and were the most stimulatory compounds tested as exogenous energy sources for motility. These values were all significantly ($P = 0.05$) greater than the control value of 4,715, as determined by FLSD test. Both asparagine and sodium citrate were later determined to be chemoattractants (unpublished data), so they could not be used for future chemotaxis experiments. Glycerol proved to be a nonattractant and stimulated motility nearly as well as asparagine at $10^{-2}$ M (unpublished data). Therefore, glycerol was incorporated at $10^{-2}$ M as an energy source into motility and chemotaxis media in future experiments.

**Chemotaxis toward leaf extracts.** Leaf inoculation with the wild-type strain resulted in typical disease symptoms on cultivar Wells II and in the hypersensitive resistant reaction on cultivar Williams several days after inoculation. Wild-type cells of *P. s. glycinea* were equally attracted to leaf extracts of both Wells II and Williams soybeans as determined by capillary assay. The number of cells that accumulated were 70,208 and 69,063 cfu per capillary for leaf extracts of Wells II and Williams, respectively. Control capillaries contained 19,553 cfu per capillary. According to FLSD test, the two treatment means are not significantly different from each other but both are significantly different from the control (FLSD = 10,654).

**Comparison of wild-type cells with nonmotile and motile revertant mutants.** All five nonmotile mutants and their motile revertants were pathogenic and able to grow on minimal medium. Average colony diameters on SMKB ranged from 1.4 to 2.1 mm for the nonmotile mutants and from 11.0 to 19.7 mm for their motile revertants after 24 hr of incubation at 25 ± 5 C. The wild-type and MR7 average colony diameters were 13.0 and 13.9 mm, respectively, after 24 hr. Isolates Nm7 and Mr7 were selected for further experiments since the spreading ability of Mr7 was similar to that of the wild-type on MKBB.

Isolates Nm7, Mr7, and wild-type all produced typical water-soaked lesions on susceptible soybean leaves upon stab inoculation and were equally pathogenic, causing identical symptoms. A hypersensitive resistant reaction was observed with all isolates on tobacco leaves. The isolates had similar generation times of 129, 136, and 113 min for wild-type, Mr7, and Nm7, respectively. These generation times do not differ significantly (FLSD test, $P = 0.05$).

Motile revertant cells exhibited active motility. The number of Mr7 cells per capillary increased significantly with increasing incubation from 0 to 5 min (Fig. 4) then increased less rapidly from 5 to 30 min, whereas the number of Nm7 cells per capillary remained at constant, low levels. All results were confirmed by microscopic examination in which cells of the Mr7 isolate were vigorously motile, whereas no motile cells were observed in suspensions of Nm7. MR7 cells were as motile and as chemotactic toward soybean leaf extract as were wild-type cells (Table 1), as determined by capillary assay. Nm7 cells were not chemotactic to soybean leaf extract.

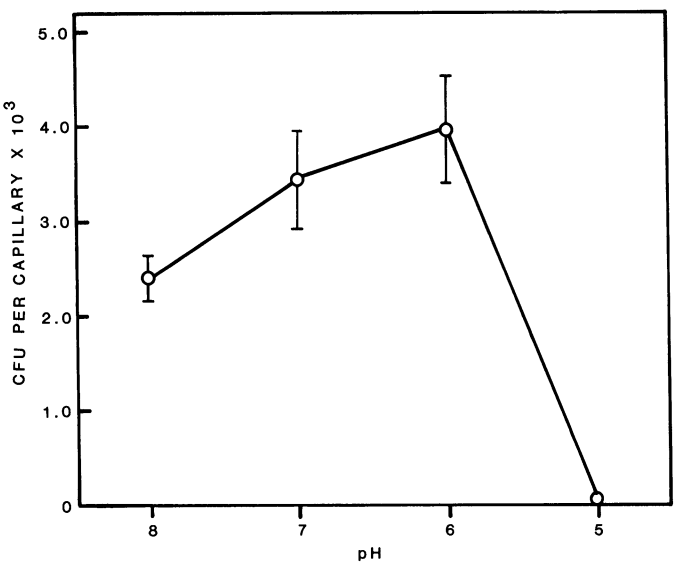


**Fig. 3.** Effect of pH on motility of *Pseudomonas syringae* pv. *glycinea* as determined by capillary assay. Assays were run for 30 min with $4 \times 10^7$ cells per milliliter suspended in $5 \times 10^{-2}$ M potassium phosphate buffer and $10^{-5}$ M ethylenediamine tetraacetic acid without glycerol. Bars represent standard error of the mean.

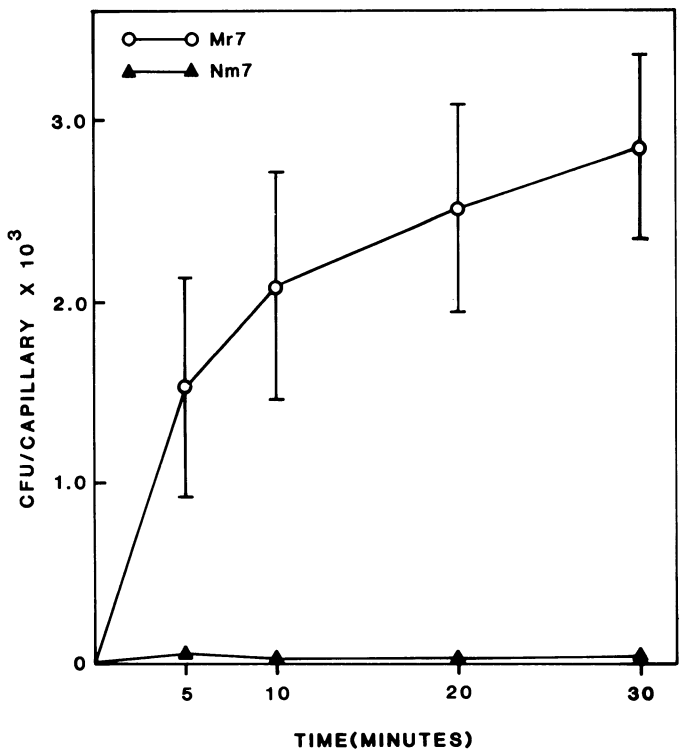


**Fig. 4.** Capillary assay for motility of motile revertant (Mr7) and nonmotile (Nm7) isolates of *Pseudomonas syringae* pv. *glycinea*. Assays were run at 24 C with $4 \times 10^7$ cells per milliliter suspended in $5 \times 10^{-3}$ M potassium phosphate buffer with $10^{-4}$ M ethylenediamine tetraacetic acid and $5 \times 10^{-3}$ M $MgCl_2$ adjusted to pH 7. Bars represent standard error of the mean.

TABLE 1. Capillary assay for chemotaxis of isolates of *Pseudomonas syringae* pv. *glycinea* toward soybean leaf extract[a]

| Isolate[b] | Colony-forming units per capillary: Attractant present | Attractant absent | Relative response[c] |
|---|---|---|---|
| WT | 60,838 a[d] | 4,792 b | 12.7 |
| Mr7 | 57,063 a | 5,221 b | 10.9 |
| Nm7 | 4 | 31 | ... |
| FLSD ($P = 0.05$) | 12,639 | 1,465 | |

[a] Assays were performed for 30 min at 24 C with $4 \times 10^7$ cells per milliliter in $5 \times 10^{-2}$ M potassium phosphate buffer containing $10^{-4}$ M ethylenediamine tetraacetic acid and $5 \times 10^{-3}$ M $MgCl_2$ that had been adjusted to pH 7. Cells were grown in modified King's B broth at 24 C.

[b] WT = wild-type, Mr = motile revertant, Nm = nonmotile.

[c] Ratio of colony-forming units accumulating in the presence of attractant to that of control (attractant absent).

[d] Means with the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test (FLSD).

**Infectivity of motile versus nonmotile cells.** Motile cells (Mr7) produced 10–12 times more lesions per square centimeter than did nonmotile cells (Nm7) after soybean leaflets were immersed in inoculum for 10 min. Immersion for 0–5 min in the motile cell suspension resulted in increasing numbers of lesions per square centimeter of leaflet, whereas immersion in nonmotile cells resulted in low, constant numbers of lesions with identical treatment (Fig. 5).

## DISCUSSION

*P. s. glycinea* is most vigorously motile at lower incubation temperatures (17–27 C), which are suboptimal for growth. This is similar to results reported previously for the plant pathogen *E. amylovora* (30). *Escherichia coli, Proteus* spp., and *Salmonella* spp. are also less motile at higher temperatures, due to inhibition of flagellar synthesis (8,12,20). Conversely, *E. herbicola* appears unique in being more motile at temperatures favoring maximum growth (27–31 C) (17). Motility of *P. s. glycinea* is optimal within a pH range of 6–7, which is much narrower than the optimal range reported for *E. amylovora* of 6–9 (30). In contrast to motility medium pH, growth medium pH has a limited affect on cell motility. Although the most vigorous and the greatest number of motile cells are observed at pH 6 and 7, motile cells are seen at all pH values, suggesting that pH affects flagellar function rather than flagellar synthesis in the pH range 5–10.

Motility of *P. s. glycinea* seems to be less sensitive to lack of a chelating agent than that of other bacteria (1,25,30). Motility is enhanced by EDTA but only at a concentration of $10^{-5}$ M and only by a factor of 2 over that of the control. At concentrations higher than $10^{-4}$ M, motility is inhibited below the control value. Motility

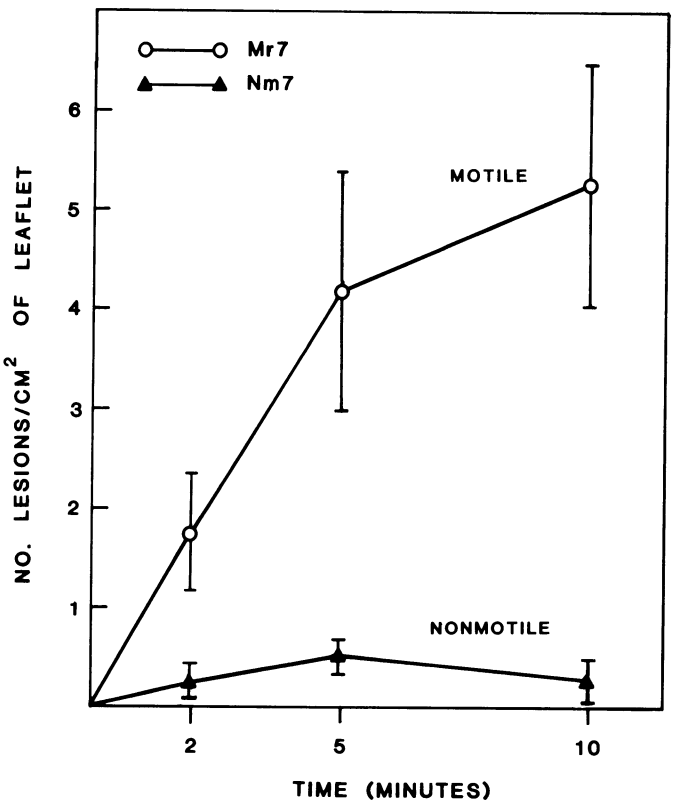


**Fig. 5.** Effect of immersion time in suspensions of motile (Mr7) or nonmotile (Nm7) cells of *Pseudomonas syringae* pv. *glycinea* on lesion development in soybean trifoliate leaflets. Suspensions were prepared by washing and resuspending cells in chemotaxis medium to a concentration of $4 \times 10^5$ cells per milliliter. Chemotaxis medium consisted of $5 \times 10^{-3}$ M potassium phosphate buffer with $10^{-5}$ M ethylenediamine tetraacetic acid, $5 \times 10^{-3}$ M $MgCl_2$, and $10^{-2}$ M glycerol adjusted to pH 6.5. Leaflets were vacuum infiltrated with distilled $H_2O$ before immersion to make them more susceptible to bacterial invasion. Bars represent standard error of the mean.

of other bacterial species generally decreases severalfold in the absence of a chelating agent (1,25,30). The $10^{-5}$ M optimum EDTA concentration for motility of *P. s. glycinea* contrasts with the optimum for motility of *E. amylovora* of $10^{-3}$ M (30), but it approximates concentrations used in experiments with other pseudomonads ($10^{-4}$ M to zero) (9,10,14,21,26). A possible explanation for this phenomenon could be that EDTA is toxic to *P. s. glycinea* at higher concentrations, thus causing the sharp decline in motility at concentrations greater than $10^{-4}$ M. Another possible explanation is that our buffer contained lower concentrations of heavy metal ions than buffer used in other experiments or that motility in this organism was less sensitive to such ions. Possibly EDTA is stimulating motility by some means other than metal ion chelation. Regardless, a very sensitive balance seems to exist between EDTA concentration and motility in *P. s. glycinea*, which merits further investigation.

Asparagine, glycerol, and sodium citrate serve as exogenous energy sources for motility in *P. s. glycinea* and stimulate motility when present in either motility or chemotaxis medium. Although motility is increased by these compounds, it can develop and persist in their absence. This is also observed in the presence and the absence of EDTA, although the presence of EDTA ($10^{-5}$ M) results in approximately twice as many cells per capillary. This is in contrast to both *E. amylovora* (30) and *Escherichia coli* (2), in which a chelating agent (EDTA) is required for cell motility in the absence of an exogenous energy source (30). In both organisms this phenomenon is due to an endogenous energy source (2).

Our study shows that *P. s. glycinea* is equally attracted to leaf extracts from a susceptible and a resistant soybean cultivar, which agrees with previous work with *P. lachrymans* on susceptible and resistant cucumber cultivars (7). These results conflict, however, with results obtained with *X. oryzae*, which is attracted to exudates from susceptible rice plants more strongly than to those from resistant rice cultivars (11). Results with *P. s. glycinea* support other data for plant-pathogenic bacteria (7,30) that suggest that chemotaxis is probably not a determining factor in pathogenicity. Most of the bacteria studied are attracted to both host and nonhost plants. Once inside plant tissue, other factors determine disease development. More research is needed with several other plant-pathogenic bacteria, however, before generalizations can be made about the role of chemotaxis in disease development.

Motile and nonmotile isolates of *P. s. glycinea* that are near isogenic are required to test the hypothesis that motile cells are more infective than nonmotile cells. The isolates Mr7 and Nm7 are as pathogenic as the wild-type on soybean leaves, induce HR on tobacco, and grow on minimal medium. This indicates that mutagenesis did not affect pathogenicity or prototrophic ability. These three isolates also possess similar generation times, which indicates that their growth rates were not affected by mutagenesis. The Mr7 isolate is as motile and as chemotactic to leaf extracts as the wild-type, whereas Nm7 is neither motile nor chemotactic. This agrees with microscopic examination, in which no motile cells were seen in Nm7 cultures while Mr7 and wild-type cultures were vigorously motile.

Infectivity is defined as the capacity of a pathogen to enter a plant and cause disease (26). This study demonstrates that motile cells of one isolate of *P. s. glycinea* have a greater infection potential than nonmotile cells on susceptible soybean leaves (Wells II) when leaves are dipped into inoculum. Our results are similar to those obtained from *P. phaseolicola* (26), in which motile cells caused approximately 10–12 times as many lesions on susceptible leaf tissue as nonmotile cells. Similarly, motile cells of *E. amylovora* (4) were more infective than nonmotile cells.

We conclude that motility assists cells of *P. s. glycinea* in causing disease. Our findings substantiate the results of Panopoulos and Schroth (26) and Bayot and Ries (4) showing that motility enhances the invasiveness and hence the infection potential of plant-pathogenic bacteria. Our finding that motility is increased by cool conditions may complement the fact that this organism is a cool-weather pathogen that causes disease and spreads best under cool, wet conditions (32). Additional research is needed in this area to determine whether there is a difference in the attraction of this

organism to wounds versus natural openings (i.e., stomates) and to determine what components of leaf extract serve as attractants. More research is also needed to determine whether motility in other bacterial plant pathogens affects disease development and to determine the exact role chemotaxis plays in the disease process.

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