Swarming and Swimming Changes Concomitant with Phase Variation in *Xenorhabdus nematophilus*

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Xenorhabdus spp., entomopathogenic bacteria symbiotically associated with nematodes of the family *Steinernematidae*, occur spontaneously in two phases. Phase I, the variant naturally isolated from the infective-stage nematode, provides better conditions than the phase II variant for nematode reproduction. This study has shown that *Xenorhabdus* phase I variants displayed a swarming motility when they were grown on a suitable solid medium (0.6 to 1.2% agar). Whereas most of the phase I variants from different *Xenorhabdus* spp. were able to undergo cycle of rapid and coordinately population migration over the surface, phase II variants were unable to swarm and even to swim in semisolid agar, particularly in *X. nematophilus*. Optical and electron microscopic observations showed nonmotile cells with phase II variants of *X. nematophilus* F1 which lost their flagella. Flagellar filaments from strain F1 phase I variants were purified, and the molecular mass of the flagellar structural subunit was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be 36.5 kDa. Flagellin from cellular extracts or culture medium of phase II was undetectable with antiserum against the denatured flagellin by immunoblotting analysis. This suggests that the lack of flagella in phase II cells is due to a defect during flagellin synthesis. The importance of such a difference of motility between both phases is discussed in regard to adaptation of these bacteria to the insect prey and the nematode host.

The genus *Xenorhabdus* (of the family *Enterobacteriaceae*) consists of the specific bacterial symbionts of the entomopathogenic nematodes of the family *Steinernematidae* (29) and was separated from the genus *Photorhabdus*, which consists almost entirely of symbionts of the entomopathogenic nematodes of the family *Heterorhabditidae* (11). The nematodes provide protection and transport for their bacterial symbionts carrying them monoxenically in a special vesicle of infective stage (L3 juveniles) of *Steinernematidae* nematodes (8) and throughout the whole intestines of *Heterorhabditidae* nematodes (17). These bacteria are transported by their nematode hosts into the hemocoel of the insect prey, inducing a lethal septicemia. The nematodes provide protection for their symbionts against the host's immune response. During the early stage of the infection, L4 and adult steinernematids produce an inhibitor of the insect-inducible immune protein (10, 18) and, for some species, axenic nematodes are pathogenic (15). The bacterial symbionts also contribute to the symbiotic relationship by establishing and maintaining suitable conditions for the nematode reproduction (26).

During in vitro culture or mass rearing of nematodes, *Xenorhabdus* and *Photorhabdus* strains spontaneously produce two colony form variants (2) which have been called phase variants (9). Only the phase I variant is isolated from infective-stage nematodes. The two variants of the bacteria are equally pathogenic to insects. However, phase I variants have been reported to provide a better support for the nematode growth than phase II variants (2). Infective juvenile nematodes resulting from natural parasitism in insects or from in vitro cultures in the presence of both variants contain only phase I variants (2). The two variants can be distinguished by several charac-

teristics. Phase I variants of *Xenorhabdus* spp. adsorb dyes on agar plates, produce phospholipase (lecithinase), and have protoplasmic paracrystalline inclusions (12), whereas these properties are either apparently absent or greatly reduced in phase II variants (9). Phase I variants also produce chemical, agar-diffusible antibiotics that minimize the secondary invasion of the cadaver by other microorganisms, while phase II variants produce no such compounds (3). However, it is clearly demonstrated that phase change occurs during the stationary period of growth and that the extent of phase variation is highly variable and not well controlled (10).

When grown on suitable solid medium, we observed for the first time a swarming behavior in *Xenorhabdus* spp. Swarming is a specialized form of bacterial surface translocation on solid media which has been observed in gram-negative and gram-positive genera including *Proteus*, *Vibrio*, *Bacillus*, *Clostridium*, and *Serratia* (6) and, more recently, in *Escherichia coli* and *Salmonella typhimurium* (19). Coordinated migration of the swarm cell population away from the central colony is a universal feature of bacterial swarming behavior. It is important to differentiate swarming from other forms of surface motility, since the term swarm zone has often been used to describe the spreading area of bacterial cells which results from normal swimming motility of bacteria on semisolid media. In fact, genuine swarming occurs on surfaces which do not allow swimming by nonswarming motile bacteria. Recently, this developmental process has been studied as a model of differentiation and multicellular behavior (6).

In the present study, we have examined phenotypically the motility, including swarming behavior, of both variants in the genus *Xenorhabdus*, and by morphological and biochemical approaches, we observed that phase II variants of *Xenorhabdus nematophilus* F1 were unable to synthesize flagella.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The sources of bacterial isolates are given in Table 1. Bacterial strains were taken from our laboratory

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TABLE 1. Sources of *Xenorhabdus* strains used in this study

Bacterial strain	Nematode host	Geographic origin
<i>X. nematophilus</i> F1	<i>Steinernema carpocapsae</i>	France
<i>X. nematophilus</i> A24	<i>Steinernema carpocapsae</i>	Russia
<i>X. nematophilus</i> AN6 (= ATCC 19061)	<i>Steinernema carpocapsae</i>	United States
<i>X. nematophilus</i> NC116	<i>Steinernema carpocapsae</i>	United States
<i>X. bovienii</i> Si	<i>Steinernema intermedia</i>	United States
<i>X. bovienii</i> SK2	<i>Steinernema kraussei</i>	Czech Republic
<i>X. bovienii</i> F5	<i>Steinernema feltiae</i>	France
<i>X. bovienii</i> F3	<i>Steinernema affinis</i>	France
<i>X. poinarii</i> G6	<i>Steinernema glaseri</i>	United States
<i>X. beddingii</i> Q58	<i>Steinernema</i> sp.	Australia

collection. Strains A24, NC116, AN6, Si, SK2, and G6 were initially provided by R. J. Akhurst (CSIRO, Canberra, Australia), and strains F1, F3 and F5 were provided by E. Bonifassi (INRA, Antibes, France). For each strain, we used phase I and phase II variants. Phases I and II of *Xenorhabdus* spp. are indicated by addition of the suffixes 1 and 2, respectively, to the strain designations, as previously described (9). For each subculture, phase status was identified by the differential adsorption of dye when the strains were grown on NBTA (nutrient agar supplemented with 25 mg of bromothymol blue and 40 mg of triphenyltetrazolium chloride per liter) or when they were grown on MacConkey agar (bromothymol blue and neutral red adsorption, respectively) and by measuring antimicrobial activity against *Micrococcus luteus* (from the Institut Pasteur Paris culture collection). Phase I colonies are blue on NBTA and produce agar-diffusible antibiotics, while phase II colonies are red and produce very little or no antibiotic.

In order to obtain phase revertants, strains A24/2, F1/2, and NC116/2 were grown for 24 h to 6 days in unshaken Luria broth (LB) and were daily spread on NBTA agar plates. Blue colonies were screened and were tested for antimicrobial activity and motility (see below).

Analysis of swimming and swarming motility. Swimming motility was observed on LB with 0.35% agar (Difco). This medium is referred to below as swim agar. To test factors inducing swarming motility, different concentrations (0.5 to 1.5%) of agar (Difco) were used in LB and in nutrient broth (NB) (Diagnostics Pasteur [5 g of peptone, 2 g of yeast extract, 1 g of meat extract, 5 g of NaCl per liter]; Biokar Diagnostics [10 g of tryptone, 5 g of meat extract, 5 g of NaCl per liter]).

Flagellum preparations. Purified flagella from strain F1/1 were prepared according to the method described by Alberti and Harshey (4) and were modified as follows. A 24-h-old bacterial culture was inoculated in the center of each of 10 plates containing 0.8% LB agar. After incubation (28°C, 20 h) bacteria were scraped from the plate surface and cells were washed with 0.1 M Tris-hydrochloride buffer (pH 7.5) plus 1 mM phenylmethylsulfonyl fluoride. The suspension was vigorously vortexed (3 min), and cell bodies were separated from the supernatant containing free flagella by a low-speed centrifugation in a Beckman JS13.1 rotor (10,000 × g, 10 min). The flagella were pelleted by high-speed centrifugation of the supernatant (76,000 × g, 40 min) in a Beckman SW 28.1 rotor and then resuspended in 0.1 M Tris-hydrochloride buffer (pH 7.5) plus 1 mM phenylmethylsulfonyl fluoride.

Electron microscopy. Electron microscopy was performed using a JEOL 1200X transmission electron microscope. A drop of swimming cells growing on swim agar was gently mixed with modified Karnofsky fixative (2.5% [vol/vol] glutaraldehyde, 1% [vol/vol] paraformaldehyde diluted with 0.15 M sodium cacodylate buffer [pH 7.4]) for 10 min. Carbon-coated copper grids (400 mesh) were floated onto a drop of fixed cell suspension (10 min) and were then rinsed in ultrapure-grade water and negatively stained with 0.5% (wt/vol) phosphotungstic acid (5 to 10 s). Purified flagella were deposited on collodionated carbon-coated copper grids (400 mesh) and were negatively stained as previously described.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lysates of whole cells and intracellular protein, as well as culture supernatant, were processed as follows. Whole-cell lysates (containing intracellular flagellin plus free flagella) were prepared by boiling cells in Laemmli buffer (21) from a 24-h-old culture grown on swim agar. Lysates of sonicated deflagellated cells (obtained from flagellum preparation [see above]) were centrifuged (16,000 × g, 15 min), and the supernatant was used as intracellular protein extracts. To check the absence of flagellin in phase II culture medium, cells were grown in nutrient broth (20 h, 28°C); to remove bacterial cells after centrifugation (9,000 × g, 15 min), ammonium sulfate was added to the supernatant to achieve 80% (wt/vol) saturation. A dialyzed fraction of the pellet was used as concentrated culture supernatant. A 10-μg amount (determined by the bicinchoninic acid protein assay [Pierce]) of each sample boiled in Laemmli gel loading buffer per lane was loaded (except for concentrated culture supernatant, for which only 2.5 μg was analyzed).

SDS-PAGE was carried out by the method described by Laemmli (21) with

12% polyacrylamide resolving gels. Protein bands were stained with Coomassie brilliant blue (Sigma).

Immunological techniques. Polyclonal antiserum was prepared against the purified and denatured 36-kDa flagellin protein of strain F1/1. As analyzed by SDS-PAGE, the 36-kDa flagellin was free of contaminants. Purified proteins (280 μg) were electrophoresed, blotted onto nitrocellulose (Schleicher & Schuell BAS85), and stained with Ponceau red (Sigma). Nitrocellulose membrane pieces containing flagellin protein were homogenized and injected into New Zealand White rabbits (specific pathogen free) by Agrobio (Villeny, France). Antiserum was collected 2 weeks after the final injection. Immunoblots were performed as follows. Electrophoretic transfer of proteins to nitrocellulose membrane (Schleicher & Schuell BAS85) was made as described by Towbin et al. (30). Nitrocellulose filters were incubated in a 1:500 dilution of rabbit antiserum directed against the denatured 36-kDa flagellin protein. At this concentration of anti-flagellin antiserum, a background hybridization to four proteins was observed. These four bands were also detected by preimmune serum. After this, anti-flagellin antiserum was absorbed with an intracellular protein extract of F1/2. This specific antiserum was used throughout the present study. Nitrocellulose membranes were probed with goat anti-rabbit immunoglobulin G conjugated with peroxidase (Sigma) by using hydrogen peroxide and 3-amino-9-ethyl carbazol as the substrate.

RESULTS

Behavior of phase variants of *X. nematophilus* F1 on solid agar. The behavior of phase I and II variants of strain F1 was examined on solid media (LB and NB) containing different agar concentrations. F1/1 cells produced a swarming colony when inoculated at the center of plates containing 0.6 to 1.2% agar with all media tested. With higher concentrations, no bacterial migration across the agar surface was observed, except when freshly poured plates were still wet. With 0.5% agar, the cells were able to move over the surface and inside the agar. Figure 1a shows the morphology of a 24-h-old colony on a 1% agar plate. With this concentration of agar, the periodic cycles of population migration (clear zones) and cell division (dark zones) were easily visualized. The complex swarming pattern exhibited dendritic branching colonies (Fig. 1a). There were different designs from plate to plate in the observed patterns. The highest surface translocation velocity was obtained with 0.8% agar at 28°C. LB medium containing 0.8% agar is referred to as swarm agar and was chosen to test other *Xenorhabdus* strains. In contrast, F1/2 taken from our collection showed a complete lack of swarming ability (Swr⁻) on all solid media tested (Fig. 1b). In order to test the loss of swarming motility during phase variation and not from phase II variant subcultures, F1/1 cultures were grown for 24 h to 7 days in unshaken LB medium, and the cultures in which phase variation occurred were spread on NBTA plates with 1% agar. All F1/1 clones exhibited blue swarming colonies, whereas F1/2 clones exhibited nonswarming red smooth colonies (data not shown).

Analysis of lack of *X. nematophilus* F1/2 swimming and swarming abilities. (i) Lack of motility. One of the principal losses leading to a lack of swarming may be a defect in swimming motility. Phase variants were inoculated near the center of a swim agar plate and were incubated at 28°C for 20 h or longer. With 0.35% agar, phase I colonies exhibited a spreading area of cells, which resulted from normal bacterial swimming motility in semisolid medium (Swm⁺) moving through water channels in agar. Typical chemotactic rings were observed (data not shown). On the contrary, phase II variants of F1 failed to migrate in swim agar (Swm⁻). This lack of swimming motility should be correlated either to a loss in chemotactic ability (Che⁻), to the flagellar ability to rotate (Mot⁻), or to a failure in flagellar synthesis (Fla⁻). When motility was examined several times and with different observation fields by light microscopy, all F1/2 cells were always nonmotile.

(ii) Lack of flagella. Since no motility was seen with phase II variants, these cells must be considered either Fla⁻ or Mot⁻.

FIG. 1. Behavior of *X. nematophilus* F1 variants on 1% LB agar. (a) Swarming colony of F1/1; (b) colony of F1/2.

In order to distinguish between these two possibilities, electron microscopy was used to search for flagella. A large proportion of *X. nematophilus* F1/1 cells grown in swarm agar plates had peritrichously arranged flagella. Electron micrography (Fig. 2a) showed flagella in several bundles (arrows in Fig. 2a). However, it is necessary to point out that F1/1 morphology (cell size and number of flagella) was generally variable in liquid media. On swarm agar, F1/1 cells also showed pleiomorphic morphology. On the other hand, the cell morphology of phase II variant cells was homogeneous and rod shaped, but no flagella were ever observed (Fig. 2b). Therefore, F1/2 cells were definitely Fla⁻.

(iii) Lack of flagellin synthesis. Immunoblotting was used in order to analyze the levels of flagellin in both variants of F1. Flagella were prepared to obtain a specific antiserum. Electron

microscopy of F1/1-purified flagella revealed highly concentrated flagellar preparations with no particulate contamination (Fig. 3b), although a few phage tail-like bacteriocin elements were occasionally seen. Electrophoretic analysis of these preparations showed one band with an apparent molecular mass of about 36.5 kDa for the flagellin structural subunit (Fig. 3a, lane 3). As expected, phase II variant preparations showed neither flagellar filaments nor denatured flagellin (Fig. 3a, lane 2).

Antiserum generated against the 36.5-kDa denatured flagellin used in this study was first absorbed with an intracellular protein preparation of F1/2. Immunoblots showed no difference in labeled 36.5-kDa band signal intensity with absorbed antiserum or with nonabsorbed antiserum, whereas the background hybridization signal decreased considerably (data not shown). The amount of flagellin was determined by immunoblotting of whole-cell extracts (flagellin plus flagella) and of intracellular protein extracts from deflagellated cells (intracellular flagellin only) of both F1 variants. Figure 4 shows that neither flagellin derived from flagella (Fig. 4b, lane 2) nor intracellular flagellin (Fig. 4c, lane 4) was detected in F1/2 cells. Moreover, no hybridization signal was obtained with concentrated phase II culture supernatant.

Swarming or swimming motility in phase variants of other *Xenorhabdus* spp. The behavior of representative strains of *Xenorhabdus* species on swim and swarm agars was examined (Table 2). The phase I variants of most strains tested were found to be Swm⁺ and Swr⁺ (except G6/1 and SK2/1, which were only Swm⁺). *Xenorhabdus bovienii* strains required lower temperature (about 24°C) than *Xenorhabdus nematophilus* strains to display reproducible swarming ability. Among strains of the same species, it was noticed that characteristics of the macroscopic appearance of swarming patterns were closely related.

Loss of swarming and swimming abilities with phase II variants was apparently a general feature in the species *X. nematophilus*; all tested phase II variants were nonmotile. Phase revertant experiments with *X. nematophilus* showed that all characteristics, including the motility system, were restored in all A24 revertants and in some F1 revertants (50%) and were partially restored in NC116 revertants (Table 2) for which only motility was observed when bacteria were grown in broth. The 50% of nonswarming and nonswimming F1 revertants (called pseudorevertants) showed no motility in broth, although they restored other phase I characteristics (Table 2). NC116 revertants produced no zone or a very small zone of migration from the site of inoculation on swim and swarm agars, and the colonies showed in-place swarming movement under the microscope.

The loss of motility was also observed with the phase II variants of *X. bovienii* (Table 2), with the exception of F3/2, which showed arborescently branching swarming colonies on swarm agar. However, no correlation between phase status and motility changes (Table 2) was observed with *Xenorhabdus beddingii* and *Xenorhabdus poinarii* strains. Table 2 also shows that phase status is difficult to define with these species. *X. beddingii* Q58/1 produced a weak amount of diffusible antimicrobial compounds, whereas other phase I variants that were tested were always strong antibiotic producers. *X. poinarii* G6 variants cannot be distinguished by adsorption of bromothymol blue and antimicrobial activity. Adsorption of neutral red was the only useful indicator to discriminate G6 variants, and even by this method, G6/1 adsorption was weak (Table 2).

FIG. 2. Morphology of *X. nematophilus* F1 variants grown on swarm agar; electron micrographs of swarm cell of *X. nematophilus* F1/1 showing numerous flagella (a [arrows]) and of F1/2 without flagella (b). Bars, 2 and 1 μm , respectively, for panels a and b.

DISCUSSION

In the present study, we examined the motility of both phases of *Xenorhabdus* spp. and found that most phase I variants exhibit a swarming behavior. This particular bacterial surface translocation phenomenon has been widely described for closely related enteric bacteria such as *Proteus* and *Serratia* spp. and for more distant bacteria such as *Vibrio* spp. (for a review, see reference 6). It is not possible to detect this phenomenon in *Xenorhabdus* spp. when they are grown on normal solid media. We showed that the swarming behavior of *X. nematophilus* depends critically on the concentration of agar in the medium. Like *Serratia marcescens* (4), *Xenorhabdus* spp. require low agar concentrations (about 0.8%), whereas an agar concentration of 2% allows swarming behavior for *Proteus* or *Vibrio* spp. (6). During this phenomenon, *Proteus mirabilis* exhibits cycles of cell differentiation-dedifferentiation. The differentiated swarm cells (size of up to 80 μm , multinucleate, aseptate, and possessing hundreds of flagella) migrate coordinately, stop, and revert by cell division into vegetative cells (this last process has been termed consolidation); differentiation is then reinitiated, and swarming continues in regular cycles of population migration and consolidation until the agar surface is covered by concentric rings formed by the swarming bacterial mass (31). The macroscopic swarming pattern of *X. nematophilus* also showed concentric zonation (Fig. 1a). We are currently examining the differentiation of swarmer cells in *X. nematophilus* F1/1 to discover if morphogenesis changes take place during *Xenorhabdus* swarming. However, this question is awkward because of the pleiomorphic aspect of phase I

FIG. 3. Purified flagella of *X. nematophilus* F1. (a) Analysis of purified protein by SDS-PAGE (12% polyacrylamide) stained with Coomassie brilliant blue. Lanes: 1, standard proteins (Pharmacia), with molecular size markers in kilodaltons indicated on the left (phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α -lactalbumin); 2, attempts to obtain F1/2 flagellar preparation undertaken in the same way as those for F1/1; 3, purified F1/1 flagella (2 μg) denatured in Laemmli (21) gel loading buffer. (b) Electron micrograph of purified F1/1 flagella negatively stained with 0.5% (wt/vol) phosphotungstic acid and observed by electron microscopy (bar, 0.2 μm).

FIG. 4. Western blot (immunoblot) analysis of flagellin synthesis in *X. nematophilus* F1 variants. Uppercase letters, SDS gel patterns stained with Coomassie brilliant blue; lowercase letters, immunoblots of corresponding gels after immunoreaction with absorbed antiserum against the 36.5-kDa denatured flagellin. The arrow points to flagellin. Lanes: 1, purified flagellin of F1/1; 2, whole-cell protein extract of F1/2; 3, whole-cell protein extract of F1/1; 4, intracellular protein extract of deflagellated F1/2; 5, intracellular protein extract of deflagellated F1/1.

Xenorhabdus cells grown on swarm agar, as noticed in this study, and in liquid medium (10).

Purification of *X. nematophilus* F1/1 flagella suggests that the major subunit of the flagella is a single protein with an apparent molecular mass of 36.5 kDa. This flagellin size, which was estimated by SDS-PAGE, is similar to that of *S. marcescens* (variable from 36 to 39 kDa) (25).

The most important finding of this work is the demonstration that phase II variants of *X. nematophilus* F1 are not only unable to swarm (Fig. 1b) but are also not flagellated (Fig. 2 and 3a). It is obvious that flagella are responsible for bacterial motility; however, to date it is not certain that a defect in flagellum synthesis is the sole cause preventing swarming ability. O'Rear et al. (24) have found that some nonflagellate transposon mutants of *S. marcescens* can spread rapidly on the surface of 0.3 to 0.5% agar, whereas nonflagellate cells of *X. nematophilus* F1/2 were unable to migrate on semisolid agar.

Thus, other properties are required to induce a complex swarming behavior and might be impaired in phase II variants. This hypothesis is in accordance with the recovery of motile NC116 revertants that show a defective progressive swarming phenotype (Dps) (Table 2).

A considerable amount of energy is required to synthesize flagella. In *E. coli*, the amount of flagellin alone composes about 8% of the total cell protein when the flagellar operons are expressed optimally (23). It is known that *E. coli* cells lose their flagella under certain growth conditions. Flagellar synthesis is inhibited during growth on D-glucose by catabolite repression (1), and, more recently, Li et al (22) described adverse growth conditions (e.g., high concentrations of inorganic salt, of carbohydrate, or of low-molecular-weight alcohols and pressure of gyrase inhibitors) that induce a lack of flagella. However, the mechanism(s) of nonflagellation resulting from adverse conditions in *E. coli* remains unknown (27). Our results revealed that the loss of motility in *X. nematophilus* is clearly correlated with a phase shift event, since (i) all phase II variants tested are nonmotile, (ii) loss of swimming and swarming motility and a phase variation event occur simultaneously (this also demonstrates that the lack of motility does not result from having subcultured phase II variants several times), and (iii) motility is restored in most *X. nematophilus* phase revertants (Table 2). Despite these complete reversions, the recovery of some pseudorevertants from F1/2, in which the whole motility system remains switched off, could lead us to hypothesize that two or more mechanisms are implied in phase variation. If the genetic mechanism(s) of phase variation is still not understood, information is available about environmental conditions causing this phenomenon. It has been noted that nutrient stress (28) or partial anaerobic growth (10) with regard to *Xenorhabdus* spp. and osmotic stress (sodium chloride deficiency) with regard to *Photorhabdus* spp. (20) enhanced the phase shift. Like *E. coli*, *Xenorhabdus* cells seem to be able to sense changes in environmental conditions. Thus, the ability of the bacteria to shut down the costly process of flagellar synthesis under certain conditions might help them to survive. Genetic studies will be useful for understanding the mechanism of nonflagellation. We have shown that antiserum to flagellin did not detect any flagellin trace in F1/2 protein extracts (Fig. 4), demonstrating that the loss of motility in phase II variants is correlated with a reduction in the amount of

TABLE 2. Principal characteristics distinguishing phases and motility of *Xenorhabdus* strains

Characteristic distinguishing phases	<i>X. nematophilus</i> ^a												<i>X. bovienii</i>				<i>X. poinarii</i> G6		<i>X. beddingii</i> Q58	
	F1			A24			NC116			AN6	Si		SK2		F5		F3		1	2
	1	2	REV	1	2	REV	1	2	REV	1	1	2	1	2	1	2	1	2	1	2
Dye adsorption																				
BTB ^b	+	—	+	+	—	+	+	—	+	+	+	—	+	—	+	—	+	—	—	+
Neutral red ^c	+	—	+	+	—	+	+	—	+	+	+	—	+	—	+	—	+	—	W	—
Production of antimicrobial compounds ^d	+	—	+	+	—	+	+	—	+	+	+	—	+	—	+	—	+	—	+	W
Motility																				
Swarming ability ^e	+	—	D	+	—	+	+	—	Dps	+	+	—	—	—	+	—	+	+	—	+
Swimming ability ^f	+	—	D	+	—	+	+	—	Dps	+	+	—	+	—	+	—	+	+	V	+

^a A total of 10 revertants (REV) from several cultures were tested.

^b Adsorption of bromothymol blue (BTB) and reduction of TTC to formazan (+, blue colonies) on NBTA plates (see Materials and Methods); reduction of TTC to formazan without any BTB adsorption (—, red colonies).

^c Adsorption of neutral red from MacConkey agar. +, red colonies; W, weak adsorption; —, colorless colonies.

^d +, inhibition of *M. luteus* growth; —, no inhibition; W, weak inhibition.

^e +, swarming colonies observed on swarm agar plates; Dps, defective in progressive swarming (see the text); —, swarming not observed; D, positive for 50% of the revertants.

^f +, motility in swim agar plates; Dps, defective in progressive swarming (see the text); —, motility not observed; V, variable; D, positive for 50% of the revertants.

cellular flagellin. The absence of secreted flagellin in culture supernatants of phase II has also been demonstrated. These last results suggest that a defect in flagellar assembly is unlikely to explain the lack of flagella. Further investigations analyzing the level of flagellin gene regulation may help to clarify a mechanism of phase shifting in *X. nematophilus*.

Phase I variants of *Xenorhabdus* have been shown to differ from phase II variants in several characteristics, including dye adsorption, production of antimicrobial factors and phospholipase (9), and the presence of proteinaceous inclusion bodies (12). In addition, *X. nematophilus* phase I variants differ from phase II variants in their interaction with hemocytes (13, 16) and in the presence of fimbriae (14). The results of the present study show the occurrence of a new property in the genus *Xenorhabdus*, i.e., swarming behavior. We have argued that the swimming and swarming changes were linked to phase variation in *X. nematophilus*. If this linkage is less clear with the other species tested, it is obvious that there is a high variability in motility within genus (Table 2). Thus, it will be important to test every new isolate of this genus to know if this motility change is phase and/or species related.

The finding of such a difference in motility between both phases may play an important part in our understanding of the behavior of these symbionts with regard to their ecological niches. Under natural conditions, phase I is isolated from the intestines of infective-stage nematodes. The ability to generate swarm cells which could promote a rapid and coordinate migration should play a major role in promoting colonization of the nematode host and insect body cavity during parasitism. For instance, the ability of the opportunistic pathogen *P. mirabilis* to colonize the urinary tract is very likely enhanced by swarming motility per se (5, 6). The swarming behavior of phase I variants and the nonmotility of phase II variants might be an important element for the knowledge of the nematode-bacterium symbiosis. In addition, the occurrence of such a phenomenon may explain the invasive colonization by bacteria of the three-dimensional culture medium on sponge during in vitro mass production described by Bedding (7).

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