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Meggan M. Hodgson · Brandye Day · Donald J. White
Louis S. Tisa

Effect of growth conditions on the motility of *Photorhabdus temperata*

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Abstract *Photorhabdus temperata* is a bioluminescent bacterium that lives in mutualistic association with entomopathogenic nematodes of the genus *Heterorhabditis*. The bacterium exists in two morphologically distinguishable phases (primary and secondary). The swimming behavior of *P. temperata* was investigated. Both the primary and secondary variants were able to swim in liquid or semisolid media under appropriate conditions. Variation in the oxygen levels had little affect on the chemotaxis and motility of the primary form, but greatly influenced the behavior of the secondary form. Under oxic conditions the secondary form was nonmotile, but motility was induced under anoxic conditions. Several phenotypic traits of the primary form were not expressed under anoxic conditions. The constituents of the growth media affected the motility of both variants. *P. temperata* required additional NaCl or KCl for optimum motility and chemotaxis. Optimal chemotactic behavior required the presence of bacto-peptone and yeast extract in the swim-migration medium. A mutant that was isolated from the secondary form was able to swim under oxic conditions and possessed an altered salt requirement for motility.

Keywords Chemotaxis · *Photorhabdus* · Signal transduction · Environmental signals · Nematode · Biocontrol agent · Anoxic conditions

Introduction

An interesting system of insect biological control is found during the life cycle of two families of entomopathogenic nematodes, the Steinernematidae and the Heterorhabditidae (for a review, see Forst et al. 1997; Forst and Nealson 1996; Owuama 2001). These nematodes have evolved a mutualistic relationship with entomopathogenic gram-negative γ-Proteobacteria (*Xenorhabdus* and *Photorhabdus*). The genera *Photorhabdus* and *Xenorhabdus* are similar in many characteristics, but they differ in several important traits. *Photorhabdus* is bioluminescent while *Xenorhabdus* is not. Another major difference between the genera is the specificity of their nematode host: Steinernematidae for *Xenorhabdus* and Heterorhabditidae for *Photorhabdus*.

The bacteria are carried inside the gut of the non-feeding third-instar infective stage nematode. These infective juvenile nematodes invade insects encountered in the soil. After the nematodes locate a susceptible insect host, they penetrate the haemocoel and release the facultative anaerobic bacteria into the hemolymph. The bacteria replicate and produce a variety of primary and secondary metabolites. The bacteria in conjunction with the nematode induce a lethal septicemia that kills the insect host within 48 h. These bacteria produce several antimicrobial compounds (Akhurst 1982; Richardson et al. 1988), hydrolytic enzymes (Boemare and Akhurst 1988; Bowen et al. 2000; Schmidt et al 1988; Wang and Dowds 1993), and insecticidal toxins (Bowen et al. 1998; Bowen and Ensign 1998; ffrench-Constant and Bowen 2000). Several of these metabolites have been purified and their mode of action is being investigated. The insecticidal toxin aids in the host killing process, while the antibiotics inhibit secondary invaders. Besides production of antibiotics and toxins, the bacteria also generate essential growth factors for the nematode. Thus, the bacteria create an optimum environment in the insect cadaver for nematode reproduction and development, and the eventual release of infective juveniles.

Both *Photorhabdus* and *Xenorhabdus* produce two phase variants, designated primary and secondary forms, which can be distinguished by biochemical tests and colony morphology (for review, see Akhurst 1980; Boemare et al. 1997; Forst et al. 1997; Forst and Nealson 1996). The primary form is often converted to the secondary form upon prolonged culturing and occasionally in vivo. Growth in

M. M. Hodgson · B. Day · D. J. White · L. S. Tisa (✉)
Department of Microbiology, University of New Hampshire,
46 College Rd., Durham, NH 03824-2617, USA
Tel.: +1-603-8622442, Fax: +1-603-8622621,
e-mail: LST@hypatia.unh.edu

Present address:
Donald J. White
Xenogen Corporation, Alameda, CA 94501, USA

low osmotic strength medium also triggers a primary to secondary phase-shift (Krasomil-Osterfeld 1995, 1997). Secondary forms are more stable and revert less frequently. The cells of the primary form produce increased amounts of antibiotics, pigments, and extracellular proteases and lipases, and in the case of *Photorhabdus* are more strongly bioluminescent than the secondary cells. Both phases are equally pathogenic when injected into the hemolymph of larvae. Within the nematode, bacteria are predominately maintained as the primary form, and nematodes grow preferentially in association with primary-form cells. Although both phase forms have been isolated from infective juveniles of their nematode partners (Ehlers et al. 1990), the numbers of infective juveniles released from their insect host are increased with the primary-phase form. *Xenorhabdus* and *Photorhabdus* show a seven-fold and three-fold increased production of infective juveniles with the primary form compared to the secondary form, respectively (Akhurst 1980). Phase variation occurs in a wide range of microbes (for a review, see Henderson et al 1999). One potential biological role for phase variation is to provide the bacterium with a strategy for adapting to more than one particular environment. The primary forms of *Photorhabdus* and *Xenorhabdus* are required for nematode development and reproduction (Forst et al. 1997; Forst and Nealson 1996; Han and Ehlers 2001). Little is known about the role of the secondary form in nature.

Our interest in *Photorhabdus* is an extension of our studies on signal transduction and bacterial diversity. Bacteria move in a coordinated manner toward attractants and away from repellents (Blair 1995). Givaudan et al. (1995) reported that the primary form of *Xenorhabdus nematophilus* is motile while the secondary form is nonmotile because it lacks flagella. In this communication, we report that both forms of *Photorhabdus* are motile under the appropriate environmental conditions.

Materials and methods

Strains

For this study, both primary- and secondary-phase variants of *Photorhabdus temperata* [formerly called *Photorhabdus luminescens* (Fischer-Le Saux et al. 1999) and *Xenorhabdus luminescens* (Boemare et al 1993)] NC19 (ATCC 29304) were used. For some experiments, primary- and secondary-phase variants of strains K122 (Griffin et al 1991) and NC1 (Bowen and Ensign 2001) were also used. Strains NC1 and NC19 are identical but were obtained from different sources (Ciche et al 2001). For each subculture, phase status was identified by pigmentation and by differential dye absorption (Boemare et al. 1997). The latter was determined by growing the strains on NBTA (nutrient agar supplemented with 25 mg bromothymol blue and 40 µg triphenyl 2,3,5 tetrazolium chloride per liter) or on MacConkey agar (bromothymol blue and neutral red absorption, respectively). Primary variant colonies are blue on NBTA and red on MacConkey agar, while secondary variant colonies are red on NBTA and off-white on MacConkey agar. On LB medium, primary-phase-variant colonies of strain NC19 were pigmented (yellowish-orange) while secondary-phase-variant colonies were off-white.

Growth conditions

Cells were grown and maintained at 28 °C in tryptone broth consisting of 1% bacto-tryptone (Difco Laboratories) and 0.5% NaCl unless otherwise noted. In addition to tryptone medium, five other growth media were used in this study: (1) LB medium consisting of 1% bacto-tryptone, 0.5% yeast extract (Difco Laboratories) and 0.5% NaCl; (2) peptone medium consisting of 1% Bacto-peptone and 0.5% NaCl; (3) LB-peptone medium consisting of 1% Bacto-peptone, 0.5% yeast extract, and 0.5% NaCl; (4) PP3 medium consisting of 1.0% proteose peptone no. 3 (Difco Laboratories), and 0.5% NaCl; (5) LB-PP3 medium consisting of 1.0% proteose peptone no. 3, 0.5% yeast extract, and 0.5% NaCl. Doubling times were determined by measuring optical density at 600 nm (OD_{600}) or turbidity with a Klett-Summerson colorimeter. Cultures were incubated with shaking at 28 °C.

Chemotaxis and motility assays

Swimming behavior was measured by the plate migration assay. Chemotactic ability of swimming cells was measured by the use of a swim-migration plate assay (Adler 1973). In this assay, bacteria migrate in response to a gradient of amino acids created by their metabolism. Swim plates containing 0.25% bactoagar (Difco Laboratories) and growth medium were inoculated in the center with a stab of approximately 10^6 cells and incubated at 22 °C unless otherwise noted. All six swim media were tested. Optimum conditions were obtained with swim medium consisting of 1.0% peptone, 1.0% yeast extract, 0.5% NaCl and 0.25% bactoagar.

Bacterial swimming behavior was observed by phase-contrast microscopy at a magnification of 400×. The cells in these behavioral assays were suspended in chemotaxis medium (10 mM K^+ phosphate, pH 7.0, 0.1 mM K^+ EDTA, and 1 mM L-methionine) or in filtered used growth medium at an optical density of 0.1 at 600 nm.

Anoxic conditions for swim plate assays

To achieve anoxic conditions, swim plates were incubated in an anaerobic hood at 22 °C under mixed gas atmosphere (85% N_2, 10% CO_2, and 5% H_2). The diameter of the chemotactic ring was measured at different time intervals for 24 or 48 h. When incubation at temperatures other than 22 °C was required, swim plates were incubated for 48 h in Brewer's jars that were rendered anoxic by the use of gas packets (BBL GasPak Anaerobic System).

Transmission electron microscopy

Bacterial suspensions were placed on Formvar-coated copper grids, negatively stained with 1% phosphotungstic acid, and viewed on a JEOL 100S transmission electron microscope.

Mutant isolation

Mutants with enhanced motility were isolated by selection. Cells from the outer ring of a tryptone swim plate were inoculated into a fresh swim plate and incubated at 28 °C for 24 h. Cells from the resulting outer chemotactic ring were streaked on tryptone agar plates and incubated overnight at 28 °C. Single colonies were inoculated into tryptone swim plates and incubated at 28 °C for 24 h. This selection procedure was repeated several times. Mutants DWA11 and DWB13 were isolated from the wild-type primary and secondary variants, respectively, and saved for further study.

Phenotypic characterization

Dye absorption with NBTA and MacConkey media was assayed as described above. In addition, EB agar (eosin Y and methylene blue

at 400 and 65 mg/l, respectively, in 2% PP3 agar) was used. Hemolytic activity was determined by observing a clearing surrounding the bacterial colonies cultured on blood agar. Lipase activity was tested on spirit blue agar containing 0.5% (v/v) Tween 20, Tween 40, Tween 60, or Tween 80. Catalase activity was determined by the addition of 10 µl of 30% H_2O_2 to isolated colonies on LB or PP3 agar. Protease activity was determined by the gelatin assay (Boemare et al. 1997). DNase activity was determined on DNase test agar containing methyl green. Antibiotic activity was evaluated by placing a 5-mm-diameter plug, taken 5 mm away from confluent growth of a 96-h culture of *P. luminescens* on PP3 agar, onto a plate of antibiotic medium 3 (Difco) that had been inoculated with *Micrococcus luteus* cells.

Biochemical traits were also measured by use of BIOLOG plates. Ten ml of peptone-yeast extract broth were inoculated with single colonies and the cultures were incubated overnight at 28 °C. Cells were harvested, washed twice with 0.85% KCl, and finally suspended in 0.85% KCl to an OD_{590} of 0.25. Washed cells (150 µl) were added to each well of a BIOLOG ECO plate. The plates were incubated at 28 °C and color formation was measured at 24 and 48 h by the use of an ELISA plate reader.

Protein profiles

Cells were grown in LB-peptone broth at 28 °C until the cultures reached an OD_{600} of about 1.0. The cells were harvested, resuspended in Laemmli sample buffer to an equivalent OD_{600}, and the samples were prepared by boiling in SDS sample buffer for 5 min. SDS-PAGE was carried out as described by Laemmli (1970). The gels were stained with Coomassie to visualize protein bands.

Results

Swimming ability and the effect of O_2

The plate migration assay was used to investigate the swimming behavior of *P. temperata* phase variants (Fig. 1). Since *Photorhabdus* is a facultative anaerobe, the effect of oxygen on chemotaxis and swimming movement was tested. The primary-phase cells formed spreading rings under oxic or anoxic conditions, indicating that the cells were motile and chemotactic (Fig. 1a, b). Secondary-phase cells formed spreading rings under anoxic conditions (Fig. 1d), but failed to spread when oxygen was present (Fig. 1c). Cells from the plate migration assays were also observed by phase-contrast microscopy. Primary-form cells taken from plates that had been incubated aerobically or anaerobically actively swam. Secondary-form cells taken from plates that had been incubated aerobically were nonmotile, whereas cells taken from plates that had been incubated anaerobically were actively motile and retained their motility when exposed to oxygen for short time periods. Several primary and secondary colonies from strains NC19, NC1, and K122 were tested and showed similar results (data not shown). Transmission electron microscopy revealed the presence of peritrichous flagella (Fig. 2). The secondary form only produced flagella under anoxic conditions (Fig. 2d), while the primary form produced flagella under both conditions. These results indicate that oxygen inhibits the motility of the secondary form by blocking flagella formation.

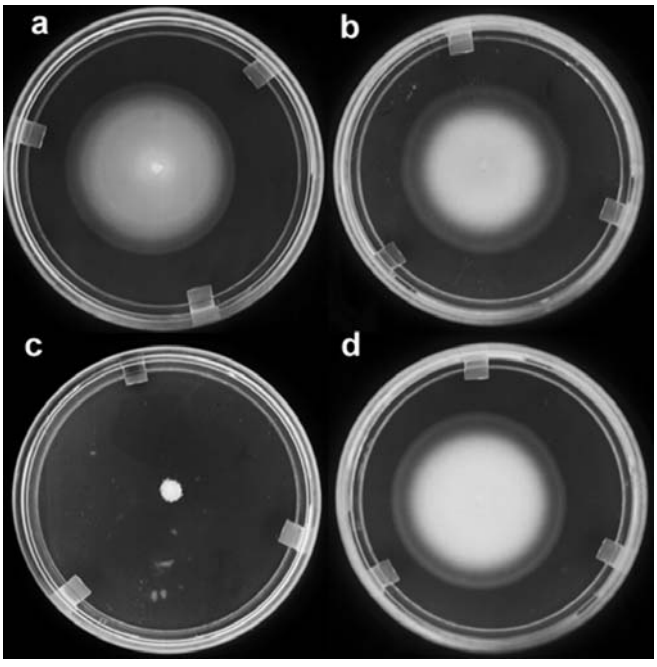

Fig. 1a–d Chemotactic properties of *Photorhabdus temperata* NC19 as determined by the swim-migration assay. Tryptone swim plates containing 0.25% bactoagar were incubated at room temperature (22 °C) for 48 h either under oxic or anoxic conditions (N_2: CO_2: H_2, 85:10:5 by volume). **a** Primary form under oxic conditions; **b** primary form under anoxic conditions; **c** secondary form under oxic conditions; **d** secondary form under anoxic conditions

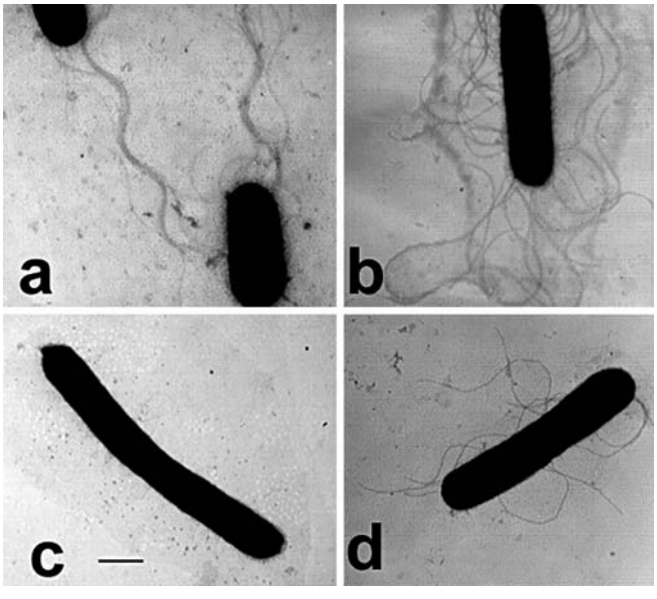

Fig. 2a–d Transmission electron microscopy of *P. temperata* NC19. Bacteria were gently placed onto Formvar-coated copper grids and negatively stained using 1% phosphotungstic acid. **a** Primary form under oxic conditions; **b** primary form under anoxic conditions; **c** secondary form under oxic conditions; **d** secondary form under anoxic conditions. *Bar* 1 µm

Table 1 Effect of media on the chemotactic properties of *Photorhabdus temperata* strain NC19. Swim migration was determined by the use of swim plates containing 0.25% bactoagar. Bacteria were grown overnight in tryptone broth and used as inocula for these experiments. The plates were incubated for 48 h at room temperature (22–24 °C). The diameters of the chemotactic rings were measured and are expressed in mm

Swim media	Swim ring diameter (mm)			
	Primary form		Secondary form	
	Oxic	Anoxic	Oxic	Anoxic
Tryptone	21.3±2.1	26.7±7.5	5.0±0.6	16.7±3.4
Tryptone+0.5% yeast extract	35.2±4.3	37.4±3.0	9.0±0.9	20.6±1.2
Tryptone+1.0% yeast extract	31.3±3.5	47.3±5.0	8.0±2.8	21.3±1.2
Peptone	52.9±3.3	64.4±2.7	6.2±0.6	30.0±1.7
Peptone+0.5% yeast extract	35.1±2.4	74.5±2.5	11.1±0.8	48.0±3.0
Peptone+1.0% yeast extract	37.0±1.9	54.0±4.9	8.7±5.3	44.0±1.6
PP3	27.3±2.3	18.0±1.7	4.0±1.0	10.7±2.5
PP3+0.5% yeast extract	35.7±1.5	32.0±3.0	10.7±2.3	33.0±3.0
PP3+1.0% yeast extract	43.0±7.5	35.0±1.7	7.0±1.4	27.0±3.0

Effect of growth media on chemotaxis

To better understand the factors influencing the motility and chemotactic behavior of *P. temperata*, the effect of growth conditions was investigated by the use of the swim-migration plate assay. The optimum temperature for swim ring formation was 28 °C for both forms (data not shown). Motility and chemotaxis were inhibited by elevated temperatures (35 °C and higher). Swim ring formation by the secondary form was only detected under anoxic conditions. Under oxic conditions, the secondary form was nonmotile at all temperatures tested.

Swim media composition greatly influenced ring formation (Table 1). Peptone resulted in larger rings than tryptone or proteose peptone. The addition of yeast extract to 0.5% stimulated swim ring formation by both forms under anoxic conditions. Elevated levels of yeast extract (1.0%) reduced the size of the primary form swim ring in peptone medium. The primary form was not influenced as strongly by yeast extract under oxic conditions, and swim ring formation was slightly inhibited by yeast extract addition to peptone media.

Effect of NaCl on motility and chemotaxis

Primary- and secondary-phase cells failed to form swim rings in LB-peptone swim agar unless additional salt was added. Addition of 86–176 mM NaCl allowed optimal swim ring formation. Phase-contrast microscopic observation confirmed that cells grown in LB-peptone without additional salt were nonmotile, whereas cells grown in the same medium with 86 mM NaCl exhibited rapid swimming motility. Transmission electron microscopy was used to demonstrate that cells grown in media without added salt lacked flagella, whereas cells grown in media with 86 mM NaCl exhibited peritrichous flagella. When peptone was replaced in the swim medium by tryptone or proteose peptone, the identical NaCl-requiring swim-migration patterns were observed for both forms (data not shown). These results suggest that medium composition did not influence the NaCl requirement for motility and chemotaxis.

Since an analysis of chemotactic behavior by use of swim ring formation is influenced by growth rate, the effect of salts on growth rates was determined. The growth rate of both phase variants was not adversely affected by the absence of additional NaCl. Cells grown in medium lacking additional NaCl grew slower, with a 1.2-fold increase in the doubling time, suggesting that the NaCl requirement for motility was not directly related to a growth rate effect.

The salt requirement for motility and chemotaxis was not specific for Na^+ (Fig. 3). At 75 mM salt concentrations, NaCl, KCl, and $MgCl_2$ restored swimming motility under anoxic conditions by the primary form to an equiva-

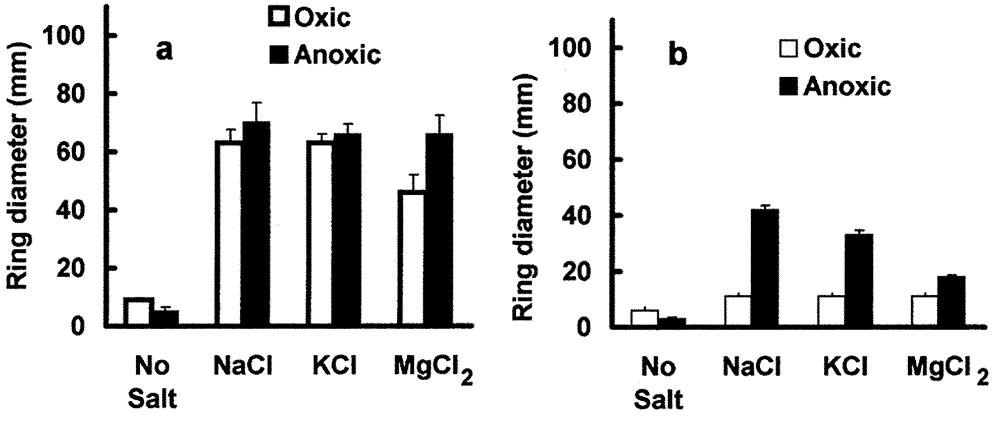

Fig. 3a, b Effect of salts on chemotactic properties of *P. temperata* NC19 as determined by the swim-migration assay. LB-peptone swim plates containing no salts or 75 mM NaCl, KCl, or $MgCl_2$ were incubated at 28 °C for 48 h. Results are shown for **a** primary-form cells and **b** secondary-form cells under oxic and anoxic conditions. Values are the average of 6–12 measurements

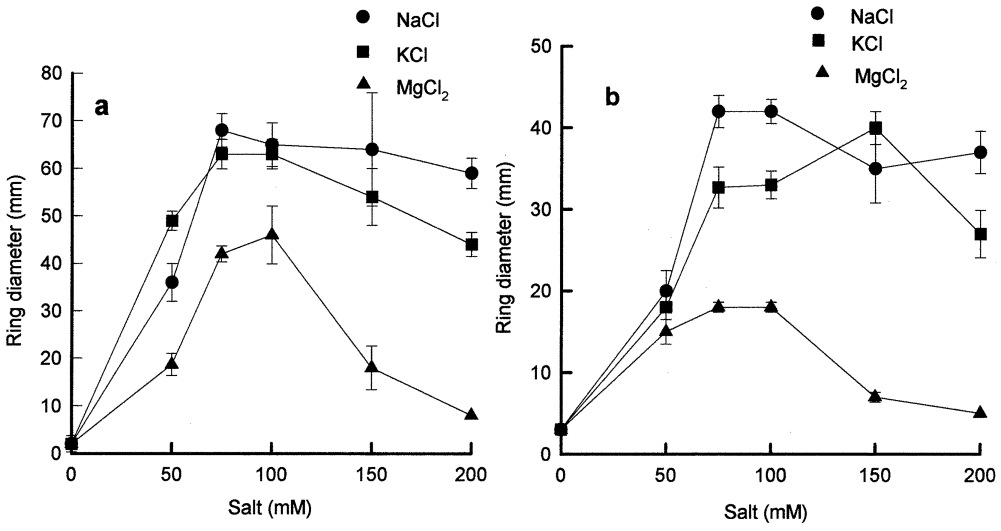

Fig. 4a, b The effect of different salt concentrations on swim ring formation by *P. temperata* NC19. LB-peptone swim plates containing different concentrations of NaCl, KCl, or $MgCl_2$ were incubated at 28 °C for 48 h. Results are shown for **a** primary-form cells under oxic conditions and **b** secondary-form cells under anoxic conditions. Values are the average of 3–6 measurements

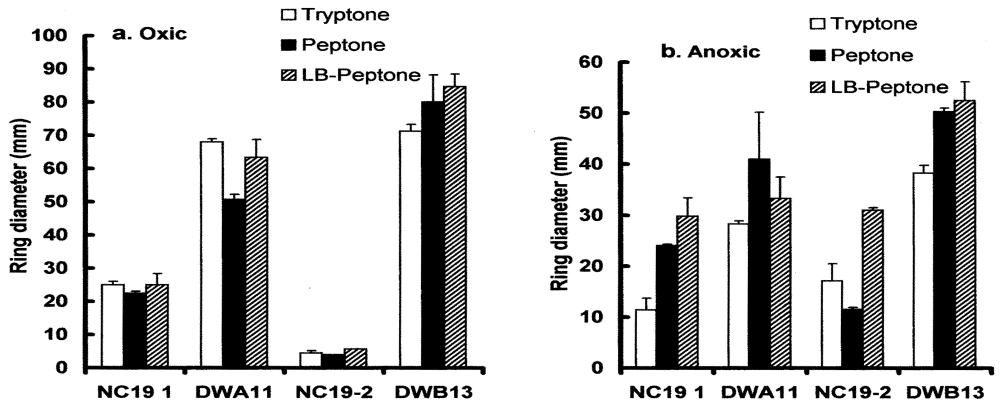

Fig. 5a, b Swim ring formation by mutants of *P. temperata* strain NC19 compared to their parental wild-types. Swim plates containing peptone, tryptone and LB-peptone media were incubated at 28 °C for 24 h. Results are shown for **a** oxic conditions and **b** anoxic conditions. Values are the average of 6–12 measurements

lent level (Fig. 3a). Under oxic conditions, there was a slight decrease in swim ring formation with $MgCl_2$ compared to the other two salts. Secondary-form cells showed the following order of preference: NaCl>KCl>$MgCl_2$ (Fig. 3b). Several primary and secondary colonies from strains NC19, NC1, and K122 were tested and showed similar results (data not shown).

A range of salt concentrations was tested and the results are shown in Fig. 4. Without added salt, swim rings were not observed. With the primary form, the addition of NaCl or KCl resulted in swim ring formation (Fig. 4a). $MgCl_2$ was not as effective as NaCl or KCl, and inhibited motility at elevated levels (>100 mM). $CaCl_2$ was unable to replace NaCl (data not shown). Similar results were observed for cells under anoxic conditions. With the secondary form, addition of NaCl resulted in the largest swim rings, followed by KCl and $MgCl_2$ (Fig. 4b). Elevated levels of $MgCl_2$ inhibited swimming motility. Secondary-form cells were nonmotile under oxic conditions.

Isolation of motility mutants and their properties

Two mutants with enhanced motility were isolated by selection as described in Materials and methods. Strain DWA11, a motility-enhanced mutant, was derived from the primary form. Strain DWB13 was derived from the secondary form and was selected for motility under aerobic conditions. Both mutants were stable and were highly motile under oxic and anoxic conditions as compared to their parental strains (Fig. 5). In contrast to its parental wild-type, oxygen did not inhibit motility by strain DWB13.

Mutants DWB13 and DWA11 did not have the same salt requirement as their parental wild-types (Fig. 6) and formed swim rings in the absence of added NaCl (ring diameters of 36.7±1.5 mm/day and 29.4±4.2 mm/day, respectively). Swim ring formation was greatest with the addition of KCl to the swim medium. Elevated levels of $MgCl_2$ inhibited swim ring formation. Motility was also inhibited by $CaCl_2$ (data not shown). Both mutant strains showed the same salt requirement patterns under anoxic conditions as was observed with oxic conditions (data not shown).

Several physiological properties of the behavioral mutants and their parental strains were investigated (Table 2). The phenotypic traits of primary-form cells under anoxic conditions differed from those exhibited under oxic conditions. Anoxic conditions down-regulated several phenotypic traits of the primary form, including hemolysin, protease, and lipase activities. Antibiotic production and catalase activity by primary-form cells were not inhibited by

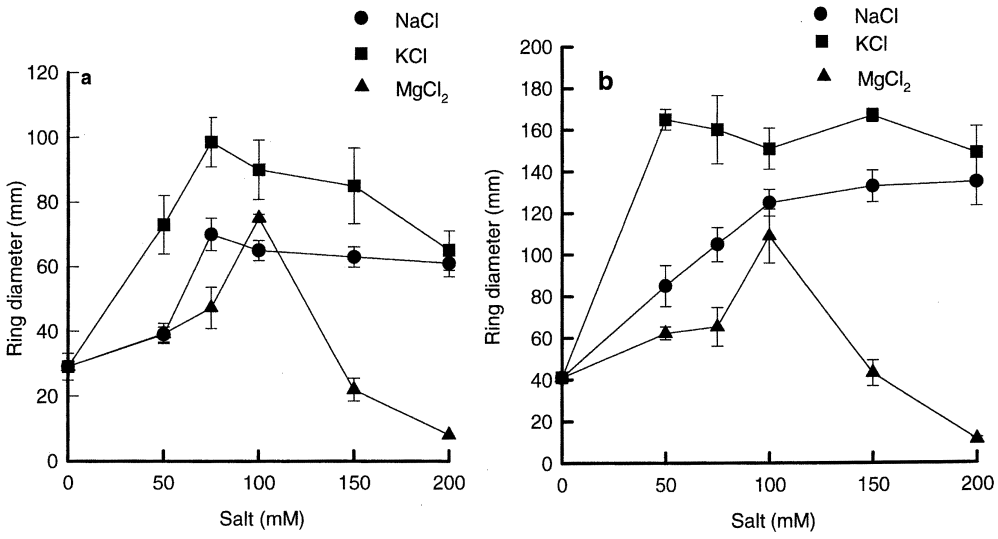

Fig. 6a, b Effect of salts concentrations on swim ring formation by the mutant strains of *P. temperata* as determined by the swim-migration assay. LB-peptone swim plates containing different concentrations of NaCl, KCl, or $MgCl_2$ were incubated at 28 °C for 48 h under oxic conditions. Results are shown for **a** DWA11 and **b** DWB13. Values are the average of 3–6 measurements

Table 2 Phenotypes of *P. temperata* mutants and their parental wild-types. Results for oxic and anoxic conditions are presented; values in parenthesis are for anoxic conditions. *1* Primary form, *2* secondary form. *w* Weakly positive. DNase and protease activities were determined by measuring the size of the halo (mm) surrounding the bacterial colony 24 h after inoculation. Strongly positive (++) >2 mm halo, positive (+) 1–2 mm halo, weakly positive (w) <1 mm halo, and negative (–) no halo. Antibiotic production was determined by measuring the size of the halo (mm) surrounding the bacterial colony 1 day after inoculation of the tester bacterium (*Micrococcus luteus*)

	NC19 1	NC19 DWA11	NC19 2	NC19 DWB13	NC1 1	NC1 2 Yellow	NC1 2 White	K122 1	K122 2
Dye absorption									
EMB	+ (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)
MacConkey	+ (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)
Extracellular products									
Lipase	+ (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)	– (–)	+ (–)	– (–)
Hemolytic	+ (–)	+ (–)	– (–)	– (–)	+ (–)	w (–)	– (–)	+ (–)	– (–)
Protease	+ (–)	+ (–)	w (–)	w (–)	+ (–)	– (–)	– (–)	++ (–)	– (–)
DNase	+ (–)	+ (–)	+ (–)	+ (–)	+ (–)	+ (–)	– (–)	+ (–)	– (–)
Antibiotics	4 (3)	2 (3)	– (2)	– (1)	2 (3)	– (3)	– (1)	8 (4)	– (2)
Pigmentation	+ (+)	+ (–)	– (–)	– (–)	+ (+)	+ (+)	– (–)	+ (+)	– (–)
Catalase	+ (+)	+ (+)	w (w)	w (w)	+ (+)	w (w)	w (w)	+ (+)	w (w)

anoxic conditions. Secondary-form cells maintained their phenotypic traits under oxic or anoxic conditions except antibiotic production. Under anoxic conditions, secondary-form cells produced antibiotics, which they did not make under oxic conditions. However, the amount of antibiotics produced by secondary-form cells was less than that produced by the primary-form cells under anoxic conditions. The primary mutant DWA11 and the secondary mutant DWB13 maintained the physiological properties of their respective forms, including dye absorption, pigmentation, and extracellular enzyme activity. These results indicate that these are true "mutants" rather than phase variants.

Biolog ECO plates were used to identify substrate utilization patterns. The primary form was capable of using six out of the 33 substrates tested (L-serine, L-threonine, pyruvic acid methyl ester, *N*-acetyl-D-glucosamine, L-asparagine and D,L-α-glycerol phosphate) while the primary mutant DWA11 oxidized only four of these six substrates. The secondary form was able to use 11 substrates (the same six as the primary form plus glycogen, ketobutryric acid, D-malic acid, Tween 40 and Tween 80), while the secondary mutant DWB13 used these 11 substrates plus hydroxybutyric acid and 4-hydroxy benzoic acid. The protein profiles of the mutants under oxic and anoxic conditions were determined by SDS-PAGE analysis and were similar to the profiles of their corresponding parental wild-type (data not shown). These results suggest that the motility mutants maintained their respective phase forms.

Discussion

The optimum conditions for motility and chemotaxis for *P. temperata* were determined in this study. Oxygen inhibited the motility of the secondary form, but had little effect on the motility of the primary form. In contrast, only the primary form of *Xenorhabdus* was capable of swimming motility (Givaudan et al 1995). To our knowl-

edge, the effect of oxygen on the motility of *X. nematophilus* has not been studied previously. Both forms of *P. temperata* also required additional salt for optimal motility. The opposite effect was observed for *X. nematophilus* (Volgyi et al 1998); the addition of >35 mM NaCl to the medium inhibited motility.

One hypothesis to explain the oxygen effect is that the secondary form is more adapted to survival in anoxic environments. The increased motility under anoxic conditions could aid secondary-form cells in migrating toward nutrients or potential terminal electron acceptors. Rosner et al. (1997) investigated the metabolism of *P. luminescens* and found no apparent differences in the fermentation metabolism between the two phase variants, but they did not explore anaerobic respiration. The secondary form grows faster and has a higher cell yield than the primary form (Beakley and Nealson 1988; Rosner et al. 1997). Smigielski et al. (1994) postulated that secondary-phase cells are better adapted to the low-nutrient conditions found in the soil, while primary-form cells are better adapted to conditions in the insect and nematode. Although there have been no reports of the isolation of *Photorhabus* from uninoculated soil, the bacteria are able to survive and grow in soil (Bleakley and Chen 1999).

The mechanism of phase variation in *Photorhabdus* is unknown (for a review, see Forst and Nealson 1996; Forst et al. 1997). An analysis of the genome structure indicates that a major DNA rearrangement or instability is not responsible for phase variation (Akhurst et al. 1992). Other studies have ruled out the loss of a plasmid or phage as the mechanism (Leclerc and Boemare 1991). One current hypothesis is that a global regulatory system controls the phenotypic traits associated with phase variation. However, the presence of intermediate forms of phase variants (Akhurst 1980; Gerritsen et al. 1992; Hu and Webster 1998) suggests that regulation is probably more complex than a simple master-switch controlling many factors. Anoxic conditions down-regulated several primary-phase traits including DNase, protease, and hemolysin activities, but antibiotic production was not oxygen-regulated with primary-phase cells (Table 2). With secondary-phase cells, anoxic conditions initiated antibiotic production and motility, but did not stimulate expression of any other primary-phase traits. Our results support the more complex model rather than the simple master-switch hypothesis. Many other environmental factors may influence this control mechanism.

Flagellum formation by *Escherichia coli* is a response to environmental stress that appears to act at the level of *flhDC* expression and is affected by catabolite repression, temperature, and other factors linked to the cell cycle (Blair 1995). Our results suggest that anoxic conditions globally affect gene expression in *P. temperata*. Oxygen inhibited flagellum production and antibiotic production by secondary-phase cells (Fig. 2), while the absence of oxygen down-regulated many primary-phase traits (Table 2). These effects of oxygen on *P. temperata* imply a potential role for an FNR-like regulator and/or an ArcA/ArcB sensor-regulator system. The generation of a secondary motility mutant (DWB13) supports this hypothesis. This mutant was highly motile under oxic conditions and did not require added salt for expression of motility. These results suggest a mutation in a regulatory element. Current studies are directed toward understanding how oxygen levels regulate gene expression in *P. temperata*.

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