DNA Relatedness between *Xenorhabdus* spp. (*Enterobacteriaceae*), Symbiotic Bacteria of Entomopathogenic Nematodes, and a Proposal To Transfer *Xenorhabdus luminescens* to a New Genus, *Photorhabdus* gen. nov.

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The levels of DNA relatedness for a broad sample of *Xenorhabdus* strains isolated from different species of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and from different geographical sources were estimated by the hydroxyapatite method. The level of DNA-DNA relatedness for the two phases of each isolate tested was not significantly different from 100%, demonstrating unequivocally that the phase variation demonstrated by all *Xenorhabdus* spp. is not due to contamination. The isolates of the described *Xenorhabdus* species coalesced into different DNA relatedness groups, confirming that *Xenorhabdus nematophilus*, *Xenorhabdus bovienii*, *Xenorhabdus poinarii*, and *Xenorhabdus beddingii*, defined on the basis of phenotypic differences, are valid species. The symbiont of *Steinernema intermedia* also coalesced with the *X. bovienii* isolates. This was the only symbiont of seven recently described and unnamed *Steinernema* spp. (including *Steinernema ritteri*, *Steinernema rara*, and *Steinernema anomali*) that formed a group with any of the previously described *Xenorhabdus* species; new species descriptions are required to accommodate the other taxa, but too few isolates were available to allow satisfactory descriptions of them. The DNA relatedness data also showed that the bacteria currently classified as *Xenorhabdus luminescens* are significantly different from all other *Xenorhabdus* strains. These data strongly support indications from previous studies of phenotypic characteristics, cellular fatty acids, and DNA relatedness that *X. luminescens* should be classified as a separate genus. A new genus, *Photorhabdus*, with an amended description of the type species, *Photorhabdus luminescens*, is proposed.

The genus *Xenorhabdus* (*Enterobacteriaceae*) consists almost entirely of the bacterial symbionts of entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae (3, 16, 29). The bacterial symbionts are carried monoxenically in a special vesicle in the infective stage (L3 juveniles) of members of the Steinernematidae (8) and throughout the whole intestine of infective juveniles Heterorhabditidae (14), which provide protection and transport for their bacterial symbionts. The nematodes also 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 (17). Both nematodes and *Xenorhabdus* spp. are pathogenic for most insects when they are released into the hemolymph (11, 12, 25). The bacterial symbionts also contribute to the symbiotic relationship by establishing and maintaining suitable conditions for nematode reproduction (24), providing nutrients and antimicrobial substances that inhibit the growth of a wide range of microorganisms (2, 10).

Phase variants of 21 strains of *Xenorhabdus* spp. from 14 nematode species isolated from various parts of the world were characterized biochemically and physiologically (9), and the data were analyzed by numerical taxonomy (6). On the basis of this evidence, the elevation of four subspecies of *Xenorhabdus nematophilus* to species status as *X. nematophilus*, *Xenorhabdus bovienii*, *Xenorhabdus poinarii*, and

Xenorhabdus beddingii was proposed, and an emended description of the species *Xenorhabdus luminescens* was given.

The first genotypic study of *Xenorhabdus* spp. was an investigation of DNA relatedness (18). This demonstrated that there were substantial differences among *X. nematophilus*, *X. luminescens*, and a third group that clustered about what is now known as *X. bovienii*. Furthermore, the *X. luminescens* group was shown to contain four DNA relatedness groups. Farmer et al. (16) subsequently identified a fifth DNA group in *X. luminescens* when they examined some isolates taken from clinical specimens. None of the DNA relatedness groups was described as a separate species because too few strains had been studied.

There were some apparent anomalies in the distribution of *X. luminescens* into DNA relatedness groups. The American Type Culture Collection type strain of *X. luminescens* was assigned to a different DNA relatedness group than clones apparently isolated from the same nematode species. Moreover, two clones obtained from strain 18 were also assigned to different groups. Grimont et al. (18) suggested that dimorphism detected in *Xenorhabdus* spp. (1) might be explained by some strains being "mixed cultures containing two different luminous *Xenorhabdus* species."

We undertook a study of DNA-DNA relatedness in *Xenorhabdus* spp. to test the hypothesis of Grimont et al. (18) concerning dimorphism by using defined phase one and phase two cultures. We also sought to test and possibly extend the taxonomic scheme for *Xenorhabdus* spp. described by Akhurst and Boemare (6).

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

Bacterium		Nematode host		Geographic source
Species	Strain	Species or family	Strain	
<i>X. luminescens</i>	Hb ^T (= ATCC 29999 ^T)	<i>Heterorhabditis bacteriophora</i>	Hb	Australia
<i>X. luminescens</i>	C1	<i>H. bacteriophora</i>	C1	United States
<i>X. luminescens</i>	K81	<i>Heterorhabditis</i> sp.	K81	Guadeloupe
<i>X. luminescens</i>	Meg	<i>Heterorhabditis megidis</i>		United States
<i>X. nematophilus</i>	AN6 ^T (= ATCC 19061 ^T)	<i>Steinernema carpocapsae</i>	DD136	United States
<i>X. nematophilus</i>	A24	<i>S. carpocapsae</i>	Agriotos	Russia
<i>X. nematophilus</i>	F1	<i>S. carpocapsae</i>	Plougastel	France
<i>X. poinarii</i>	G6 ^T (= UQM 2216 ^T)	<i>Steinernema glaseri</i>		United States
<i>X. poinarii</i>	NC40	<i>S. glaseri</i>	NC40	United States
<i>X. beddingii</i>	Q58 ^T (= UQM 2872 ^T)	<i>Steinernema</i> sp.	Q58	Australia
<i>X. bovienii</i>	T228 ^T (= UQM 2211 ^T)	<i>Steinernema feltiae</i>	T228	Australia
<i>X. bovienii</i>	F3	<i>Steinernema affinis</i>	F3	France
<i>X. bovienii</i>	SK2	<i>Steinernema kraussei</i>		Czechoslovakia
<i>Xenorhabdus</i> sp.	Si	<i>Steinernema intermedia</i>		United States
<i>Xenorhabdus</i> sp.	Q1	Steinernematidae	Q1	Australia
<i>Xenorhabdus</i> sp.	SaV	<i>Steinernema anomali</i>	Voronezh	Russia
<i>Xenorhabdus</i> sp.	CBG	<i>Steinernema</i> sp.	CB2B	People's Republic of China
<i>Xenorhabdus</i> sp.	W1	<i>Steinernema</i> sp.	W1	Australia
<i>Xenorhabdus</i> sp.	K77	<i>Steinernema rara</i>	K77	Argentina
<i>Xenorhabdus</i> sp.	K78	<i>Steinernema ritteri</i>	K78	Argentina

MATERIALS AND METHODS

Bacterial strains. The sources of bacterial isolates are listed in Table 1. For each strain we used two isolates (phase one and phase two cultures of the strain). Phase one bacteria were isolated directly from the infective-stage nematodes (stage L3), and phase two bacteria were isolated from in vitro cultures established with the nematodes and phase one symbionts. The phases of cultures are designated by the suffixes /1 and /2 (e.g., isolates AN6/1 and AN6/2 are phase one and phase two cultures, respectively, of strain AN6^T [T = type strain]), as previously described (9).

Bacterial DNA. DNA was extracted from all strains and phases and was purified by a modification of the Marmur method (20). The DNAs were fragmented by sonication until the average fragment size was about 0.5 kbp as assessed by gel electrophoresis. Sonicated DNA preparations were dialyzed against 0.28 M phosphate buffer (pH 7.0) and stored at 4°C with some drops of chloroform.

Labelling DNA. DNA was labelled with ³²P by nick translation by using the protocol of Maniatis et al. (23). Labelled DNA was separated from free radioactive nucleotides by overnight dialysis against 0.1× TE buffer.

DNA hybridizations. Hybridizations were carried out in Eppendorf tubes containing 30 µg of unlabelled test DNA and 0.01 µg of labelled reference DNA in 300 µl of 0.28 M phosphate buffer. The mixture was heated (100°C, 10 min), placed in ice for a few minutes, and then incubated overnight at 60°C. The molarity of the phosphate buffer was adjusted to 0.14 M immediately before 200-µl aliquots of each sample were loaded onto a hydroxyapatite chromatography column.

For each experiment, two control hybridizations were run in duplicate. The first control, containing only labelled DNA, allowed estimation of binding of the reference DNA back to itself, providing a baseline. In the second control the unlabelled DNA was taken from the same DNA sample as the reference DNA (i.e., 100% relatedness).

Hybridizations between phase one and phase two cultures of each strain were carried out. The type strains of the *Xenorhabdus* species (AN6^T, T228^T, G6^T, Q58^T, and Hb^T)

and symbionts isolated from nematode species other than the hosts of the type strains (K77, SaV, Si, K78, CBG, Q1, W1, K81, C1, and Meg) were compared with each other. Other strains of *X. nematophilus*, *X. poinarii*, and *X. bovienii* were hybridized to some members of the first set so that within-group and between-group levels of relatedness could be assessed more thoroughly.

For pairwise comparisons, reciprocal hybridizations were carried out (i.e., first one strain and then the other was used as the labelled strain). Although most pairwise comparisons were replicated, it was not logistically feasible to do multiple replications of each pairwise comparison. However, for pairwise comparisons that were critical (e.g., type strains) or for pairwise comparisons for which values that varied significantly from expectations based on phenotypic and nematode host data were obtained, multiple replications (up to eight) were performed to ensure a high level of precision.

Estimation of DNA relatedness. The microcolumn method of Lachance (22) was used to separate single-stranded DNA and double-stranded (DS) DNA at 60°C. Single-stranded DNA was eluted at a low molarity (0.14 M phosphate buffer), and then the DS DNA was eluted in 0.48 M phosphate buffer. The two samples eluted from the microcolumns were each mixed with 6 ml of aqueous scintillation fluid and counted with a Beckman model LS 2800 scintillation counter.

The proportion of DS DNA calculated from these measurements was then used to estimate the relative binding ratio (RBR) or level of relatedness between the reference strain and the test strain as follows: $RBR = 100 \times [(PT - PC1)/(PC2 - PC1)]$, where RBR is the RBR for test isolate T and the reference isolate, expressed as a percentage; PT is the proportion of DS DNA for the test-reference pair; PC1 is the proportion of DS DNA for the 100% relatedness control (mean of two replicates); and PC2 is the proportion of DS DNA for the baseline control (mean of two replicates).

Statistical analysis. Data were analyzed by Student's *t* test (27). The variation from 100% for the RBRs for each phase one-phase two comparison was tested, and the RBRs for

TABLE 2. Levels of DNA relatedness for *Xenorhabdus* strains for which all pairwise comparisons were made

Bacterium		RBR (%) with strain ^a :													
Species	Strain	Hb ^T	K81	C1	Meg	AN6 ^T	G6 ^T	T228 ^T	Si	Q58 ^T	K77	SaV	Q1	CBG	W1
<i>X. luminescens</i>	K81	63													
<i>X. luminescens</i>	C1	32	54												
<i>X. luminescens</i>	Meg	32	53	86											
<i>X. nematophilus</i>	AN6 ^T	11	14	14	16										
<i>X. poinarii</i>	G6 ^T	11	13	12	6	19									
<i>X. bovienii</i>	T228 ^T	5	13	7	10	19	28								
<i>Xenorhabdus</i> sp.	Si	7	10	7	6	32	23	64							
<i>X. beddingii</i>	Q58 ^T	8	6	15	14	31	25	27	17						
<i>Xenorhabdus</i> sp.	K77	12	12	9	11	33	23	17	29	25					
<i>Xenorhabdus</i> sp.	SaV	11	10	13	14	29	23	22	27	32	32				
<i>Xenorhabdus</i> sp.	Q1	6	19	13	23	30	17	28	33	37	24	43			
<i>Xenorhabdus</i> sp.	CBG	10	15	18	14	36	31	25	20	35	16	48	38		
<i>Xenorhabdus</i> sp.	W1	9	14	13	10	30	28	17	18	29	16	43	48	44	
<i>Xenorhabdus</i> sp.	K78	13	20	16	20	38	23	26	28	25	27	24	31	31	19

^a For each pair, the mean of RBR data for phase one and phase two cultures and for reciprocal hybridizations.

reciprocal hybridizations were compared. The same test was used to assess whether a particular RBR was significantly different from a particular RBR range (>60, >20, or <20%).

Phenotypic characterization. A total of 29 *X. luminescens* isolates were examined for the main phenotypic characteristics that distinguish *X. luminescens* from the other *Xenorhabdus* spp. (6) by using the methods of Boemare and Akhurst (9).

RESULTS

DNA relatedness. There was no significant difference ($P < 0.05$) from 100% for the relatedness of phase one and phase two cultures for any of the 20 strains examined. There was also no significant difference ($P < 0.05$) in RBR between the reciprocal hybridization data for any strain. Consequently, the data for phase one and phase two cultures of each strain and for the reciprocal hybridizations of each pair were combined for the calculation of the mean RBR for each pair of strains. The mean RBRs calculated in this way (Tables 2 and 3) were used to assess the relationships among the 20 strains examined. The data matrix is incomplete because all possible pairwise comparisons were not made. An interpretation of the data is presented as a complete matrix in Fig. 1.

The mean RBRs for all pairs of strains generally fell into three classes: >60, 20 to 60, and <20% (Tables 2 and 3). The values for reassociation between strains belonging to the same phenotypic species (e.g., *X. bovienii* T228^T, F3, and SK2) were significantly more than 60% ($P < 0.05$). The level of hybridization between an *X. luminescens* strain and a non-*X. luminescens* strain was never significantly more than 20% ($P < 0.05$). The levels of relatedness between most

non-*X. luminescens* strains were not significantly more than 20% ($P < 0.05$).

Rarely was the level of hybridization between strains belonging to different species in the range from 38 to 60%. Undescribed strains SaV, Q1, W1, and CBG seem to form an unusual group because the pairwise relatedness levels were $43 \pm 5\%$. Si was the only strain not previously classified (Tables 2 and 3) that exhibited more than 60% relatedness with a named species; its levels of relatedness with the three strains of *X. bovienii* were not significantly more than 60% ($P < 0.05$).

Phenotypic results. Phenotypic characterization of *X. luminescens* isolates from a range of *Heterorhabditis* spp. and from a range of geographical locations showed that some characteristics for which *X. luminescens* was previously described as positive (6) should now be considered variable characteristics. Only 6 of the 29 isolates produced indolpyruvic acid from tryptophan, 18 hydrolyzed esculin, and gluconate and inositol were assimilated by only 24 and 18 isolates, respectively. Lipolysis of Tween 85 occurred in 26 of the 29 isolates.

The phenotypic characteristics that are most useful for distinguishing *X. luminescens* from other *Xenorhabdus* species are presented in Table 4.

DISCUSSION

This DNA relatedness study demonstrates unequivocally that phase variation in the genus *Xenorhabdus* (1, 9) is a real phenomenon and not merely an artifact due to contamination, as has been suggested previously (18). The level of DNA relatedness between the two phases of any strain was

TABLE 3. Levels of DNA relatedness for *Xenorhabdus* strains for which supplementary comparisons were made

Bacterium		RBR (%) with strain ^a :							
Species	Strain	Hb ^T	AN6 ^T	G6 ^T	T228 ^T	Si	Q58 ^T	K77	SaV
<i>X. nematophilus</i>	A24	10	75	23	16	ND ^b	19	26	32
<i>X. nematophilus</i>	F1	4	63	20	24	24	24	27	33
<i>X. bovienii</i>	F3	9	27	28	75	68	33	27	20
<i>X. bovienii</i>	SK2	9	32	14	75	57	27	ND	22
<i>X. poinarii</i>	NC40	11	26	71	19	29	24	ND	25

^a For each pair, the mean of RBR data for phase one and phase two cultures and for reciprocal hybridizations.

^b ND, not done.

FIG. 1. Interpretation of the DNA relatedness data showing the levels of relatedness of 20 strains of bacterial symbionts of entomopathogenic nematodes.

higher than the level of DNA relatedness between two strains belonging to any one species. The experimental error inherent in the estimation of levels of DNA relatedness means that it is impossible to be certain that two samples of DNA are completely homologous. However, since the levels of DNA relatedness of phase one and phase two cultures

were not significantly different from 100%, in each strain the phases must share a common genome, or at least very similar genomes.

The validity of the species *X. nematophilus*, *X. poinarii*, *X. bovienii*, and *X. beddingii*, which were described on the basis of phenotypic characteristics (6), was confirmed. There was a strong clustering of the data, with 98 of 105 pairs of strains having levels of DNA relatedness of either >60 or <40%. Those strains that had previously been characterized (3, 9) as *X. nematophilus* (AN6^T, A24, and F1), *X. poinarii* (G6^T and NC40), or *X. bovienii* (T228^T, F3, and SK2) formed distinct groups with more than 60% internal relatedness and with less than 40% relatedness to any other strain (Fig. 1). The one strain included in this study that had previously been characterized as *X. beddingii* exhibited less than 37% relatedness with all other strains and clearly represents a distinct taxon. These data are similar to previously published data (18), except for the relatedness of *X. nematophilus* and *X. poinarii*. Suzuki et al. (28) found that strain G21 of *X. poinarii* exhibited a high degree of relatedness (66 to 106%) to five strains of *X. nematophilus*, whereas in the study reported here we found that the type strains of these species exhibited only 19% relatedness (Table 2). This latter value was corroborated by the values for relatedness between the type strains and other members of these species (Table 3). The only phenotypic data provided for the strain used by Suzuki et al. (28) was fatty acid composition, which, according to the data of Janse and Smits (21), is unsuitable for resolving differences between *X. nematophilus* and *X. poinarii*. It seems probable that strain G21 was misidentified.

Of the strains that had not been previously identified as one of the named species (with the exception of the *X. luminescens* strains), only Si could be assigned to a previously defined group. This strain clearly belongs to the *X. bovienii* DNA relatedness group, which contains the sym-

TABLE 4. Characteristics distinguishing between *X. luminescens* and *Xenorhabdus* spp. associated with members of the family Steinernematidae

Characteristic	<i>Xenorhabdus</i> species associated with members of the Steinernematidae	<i>X. luminescens</i>
Bioluminescence	— ^a	+
Catalase	—	+
Insect pathogenicity ^b	d	+
Lecithinase ^c	d	+
Urease	—	d
Indole	—	d
Utilization of:		
Arbutin	—	d
<i>N</i> -Caproate	d	—
Diaminobutane	d	—
DL-Lactate	d	—
Ribose	d	+
Trehalose	+	d
Tyrosine	d	+

^a +, >90% of the strains are positive; —, 0 to 9.9% of the strains are positive; d, 10 to 89.9% of the strains are positive.

^b The 50% lethal dose is less than 1,000 cells when the organisms are injected into the hemocoel of insects.

^c This is a phase-related characteristic (6) and is normally detected only in phase one; it was detected in phase one of 24 of 25 strains for which both phases were available. No phase one cultures could be obtained for five clinical strains of *X. luminescens* (16) included in this study; all of these strains were negative for lecithinase activity.

bionts of four nematode species, *Steinernema feltiae* (= *Steinernema bibionis*), *Steinernema kraussei*, *Steinernema affinis*, and *Steinernema intermedia*.

Six strains could not be assigned to any of the DNA relatedness groups. Strains K77 and K78 did not exhibit more than 38% relatedness with any other strain and exhibited only 27% relatedness between themselves. These strains probably represent new species. The levels of DNA relatedness for the remaining undescribed strains, SaV, Q1, W1, and CBG, range between 38 and 48%, suggesting that each of these four strains represents a distinct species. These species are more closely related to one another than any other *Xenorhabdus* species are. However, since each of these six putative species is represented by only one isolate, no satisfactory species definitions could be made.

The *X. luminescens* strains clearly form a DNA relatedness group that is distinct from all of the other *Xenorhabdus* strains. These DNA reassociation data strongly support other DNA relatedness data (18), phenotypic data (6, 9), and chemotaxonomic data (28) in showing that *X. luminescens* is significantly different from the other *Xenorhabdus* spp. In the data presented here the four *X. luminescens* strains exhibited less than 20% homology to any other *Xenorhabdus* strain, whereas the levels of relatedness between the other *Xenorhabdus* strains were generally more than 20% and up to 48%. Similarly, Suzuki et al. (28) found that the levels of DNA relatedness between *X. nematophilus* and *X. bovienii* ranged between 31 and 46%, but the levels of DNA relatedness between these species and *X. luminescens* were only 7 to 27%. Grimont et al. (18) found little difference between the levels of DNA relatedness of *X. nematophilus* and *X. luminescens* and the levels of relatedness between either of these two species and members of 73 DNA relatedness groups belonging to the family *Enterobacteriaceae*. The DNA relatedness data are also supported by 16S RNA oligonucleotide cataloging data (13, 26). The dendrogram of S_{AB} data may present unreliable estimates of relationships because of group size dependency in the sorting strategy. Examination of the basic data shows that *X. nematophilus* was as dissimilar from *X. luminescens* as it was from *Escherichia coli* (S_{AB} , 0.70) (13, 26).

X. luminescens strains are also well distinguished phenotypically from all other *Xenorhabdus* spp. (Table 4). Among the differentiating characteristics are bioluminescence and catalase activity, which are both physiologically very significant. *X. luminescens* is the only terrestrial bioluminescent bacterium, and the absence of catalase activity in the other *Xenorhabdus* spp. is very unusual for the *Enterobacteriaceae*. Since all *Xenorhabdus* strains occupy very similar environmental niches, major physiological differences are particularly significant taxonomic indicators. The differences between the two groups is also demonstrated by their interactions with nematodes. *X. luminescens* isolates do not support culture of any *Steinernema* species in vitro (4) but may support culture of nonhost *Heterorhabditis* spp. (7, 19). Similarly, the other *Xenorhabdus* spp. do not support culture of *Heterorhabditis* species (4a) but may support culture of nonhost *Steinernema* spp. (4). Further evidence of significant differences between *X. luminescens* and other *Xenorhabdus* spp. is provided by chemotaxonomic studies. Independent studies of *X. nematophilus*, *X. bovienii*, *X. poinarii*, and *X. luminescens* demonstrated significant qualitative and quantitative differences in the cellular fatty acid compositions of *X. luminescens* and the other *Xenorhabdus* species (21, 28). Phenotypically and genotypically *X. lumi-*

nescens is so distinct from other *Xenorhabdus* spp. that it should be assigned to a separate genus.

There are evidently several subgroups within *X. luminescens*. Grimont et al. (18) recognized four DNA relatedness groups. A fifth relatedness group was identified when *X. luminescens* was isolated from human clinical specimens not associated with entomopathogenic nematodes (16). The four *X. luminescens* isolates used in the study described here form at least two groups: strains C1 and Meg, with 86% relatedness, and strains Hb^T and K81, with 63% relatedness. The lower level of relatedness of the latter group and the difference in the levels of relatedness of Hb^T with C1 and Meg (32 and 32%) and of K81 with these strains (54 and 53%) indicate that Hb^T and K81 might represent two groups. Like the studies of Grimont et al. (18) and Farmer et al. (16), this study did not include a sufficient variety of *X. luminescens* isolates to allow satisfactory definition of the subgroups. A study which includes *X. luminescens* isolates from a broad range of host and geographic sources is required. Consequently, the creation of a new genus to accommodate *X. luminescens* is proposed without the resolution of the taxonomic status of the subgroups. The description below is based on the data of Akhurst (3), Farmer (15), Grimont et al. (18), Akhurst and Boemare (5), Boemare and Akhurst (9), and Farmer et al. (16) and the phenotypic data obtained in this study.

Description of *Photorhabdus* Boemare, Akhurst, and Mourant gen. nov. *Photorhabdus* (Pho.to.rhab'dus. Gr. n. *photo*, light; Gr. fem. n. *rhabdus*, rod; M.L. n. *Photorhabdus*, bioluminescent rod-shaped bacterium). Gram-negative, asporogenous rods. Cell size is highly variable within and between cultures, ranging from 2 by 0.5 to 10 by 2 μ m, with occasional filaments up to 30 μ m long; spheroplasts occur in the last stage of exponential growth. Motile and peritrichous. Most strains produced red-, pink-, or yellow-pigmented colonies, especially on rich media (tryptic soya agar, yolk nutritive agar). Facultatively anaerobic; metabolism respiratory and fermentative. Bioluminescent, usually detectable by a dark-adapted eye; bioluminescence intensity varies within and between isolates and may be detectable only with a photometer or scintillation counter in some isolates. One nonluminescent isolate is known. Catalase positive. Does not reduce nitrate. Negative for oxidase, *o*-nitrophenyl- β -D-galactopyranoside, Voges-Proskauer, arginine dihydrolase, and lysine and ornithine decarboxylase tests. Proteolytic for gelatin. Lipolytic activity on Tween 20; many strains are lipolytic for Tween 40, Tween 60, Tween 80 and/or Tween 85. Acid is produced from glucose without gas; acid is produced from very few other carbohydrates. Acid is produced from fructose, mannose, and *N*-acetylglucosamine; acid production from glycerol, ribose, and maltose is variable. Succinate, fumarate, L-tyrosine, L-glutamate, and glucosamine are utilized as sole carbon and energy sources. The optimum temperature is usually ca. 25°C; some strains do not grow at 37°C. Pathogenic for insects; the 50% lethal dose is less than 100 cells when the organisms are injected into *Galleria mellonella* hemocoel. The G+C content of the DNA is 43 to 44 mol%. The natural habitat for most strains is the intestinal lumen of entomopathogenic nematodes belonging to the family Heterorhabditidae and insects infected by these nematodes; one DNA relatedness group is associated with human clinical specimens.

Within an isolate colony morphology may be highly variable. Two extreme types are characterized as phases one and two. Phase one is found in the infective stage of the nematode host; phase two is known only from in vitro

culture and clinical specimens. Phase one cultures produce highly mucoid colonies and absorb dye, forming red colonies from MacConkey agar; they also adsorb other dyes, such as bromothymol blue and Congo red. A large proportion of cells contain proteinic inclusion bodies. Phase one cultures produce agar-diffusible antimicrobial compounds and lecithinase. Phase one is very much more bioluminescent and more lipolytic than phase two. In pigmented strains, phase one is differently pigmented than phase two. Phase two colonies are not mucoid and do not absorb dye; very weak antimicrobial and lecithinase activities may be detected. The change from phase one to phase two usually occurs during the stationary period of in vitro cultures; change from phase two to phase one has not been demonstrated. Several intermediate colony forms, possessing at least some phase one properties, have been described.

The type species is *Photorhabdus luminescens* (Poinar and Thomas 1979) Boemare, Akhurst, and Mourant comb. nov.

Emended description of *Photorhabdus luminescens* (Poinar and Thomas 1979) Boemare, Akhurst, and Mourant. Large rods (2 by 0.5 to 6 by 1.4 μm). Bioluminescence is more than 100-fold greater in phase one than in phase two. Indole reaction positive or weak in at least one phase for most strains. Citrate is utilized as a sole carbon and energy source. Acid is produced from inositol and maltose by most strains. Inclusion bodies occur in phase one cell protoplasm and are poorly produced in phase two cultures. The G+C content of the DNA is 43.0 to 44.0 mol%. The natural habitat is the intestinal lumen of entomogenous nematodes such as *Heterorhabditis bacteriophora* and insects infected by these nematodes. Type strain Hb has been deposited in the American Type Culture Collection under accession number ATCC 29999.

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