Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov.

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The taxonomic position of *Photorhabdus* strains was examined through the results of DNA relatedness (S1 nuclease method) studies associated with the determination of ΔT_m , 16S rRNA phylogenetic inferences and phenotypic characterization, including morphological, auxanographic, biochemical and physiological properties. Three genomic species were delineated on a consensus assessment. One of these species corresponded to *Photorhabdus luminescens*, since strains were at least 50% related to the type strain of this species with ΔT_m less than 7 °C. The two other species were novel genomic species II and III, which were less than 40% related to each other with ΔT_m higher than 9 °C. A comparison of the complete 16S rDNA sequences of several representatives of genomic species II and genomic species III revealed that each of them formed a stable lineage independent of the cluster generated by *P. luminescens* strains. The genomic species differed in their maximum temperatures for growth. A correlation with the ecological origin of the bacterial samples was noticed. The heat-tolerant group I (maximum growth temperature 35–39 °C) corresponded to the symbionts of *Heterorhabditis bacteriophora* groups Brecon and HP88 and *Heterorhabditis indica*, nematodes living in warm and tropical countries, respectively. Group II (maximum growth temperature 33–35 °C) encompassed symbionts from *Heterorhabditis megidis*, *Heterorhabditis zealandica* and group NC1 of *H. bacteriophora*, nematodes isolated in temperate climates. Group III were bacteria isolated from human specimens. Two new species, *Photorhabdus temperata* sp. nov. (type strain CIP 105563^T) and *Photorhabdus asymbiotica* sp. nov. (type strain ATCC 43950^T), are proposed for genomic species II and III, respectively. Species I and II can be separated into sub-groups on the basis of high DNA–DNA relatedness (more than 80% DNA binding with $\Delta T_m < 1.5$ °C), 16S rDNA branching and phenotypic characters. Therefore, we propose that the two species *P. luminescens* and *P. temperata* should be subdivided into subspecies as follows: *P. luminescens* subsp. *luminescens* subsp. nov. (type strain ATCC 29999^T), *P. luminescens* subsp. *akhurstii* subsp. nov. (type strain CIP 105564^T), *P. luminescens* subsp. *laumondii* subsp. nov. (type strain CIP 105565^T) and *P. temperata* subsp. *temperata* subsp. nov.

Keywords: DNA–DNA hybridization, ΔT_m , 16S rDNA sequencing, phenotypic data, polyphasic classification

INTRODUCTION

The symbiotic bacteria of entomopathogenic nematodes have attracted attention because they are involved in insect pathogenicity and in the development of their hosts, which are used for biological control. A high specificity exists between the bacterial symbionts and their nematode host. Symbionts belonging to the genus *Photorhabdus* are carried monoxenically throughout the whole intestine of infective juveniles of the genus *Heterorhabditis*, which in turn provide protection and transport for their bacterial symbionts. Species of the genus *Photorhabdus* are pathogenic for most insects when released into the haemolymph. The bacterial symbionts also contribute to the symbiotic relationship by establishing and maintaining suitable conditions for nematode reproduction (Boemare *et al.*, 1997) and providing nutrients and antimicrobial substances that inhibit the growth of a wide range of micro-organisms (Akhurst, 1982).

The genus *Photorhabdus* (*Enterobacteriaceae*) consists mostly of the bacterial symbionts of entomopathogenic nematodes of the family Heterorhabditidae, with some isolates from clinical sources (Farmer *et al.*, 1989). The first isolates of *Photorhabdus* were reported by Khan & Brooks (1977) and Poinar *et al.* (1977). They were characterized by bioluminescence and by their symbiotic association with the entomopathogenic nematodes *Chromonema heliothidis* (synonymous with *Heterorhabditis bacteriophora*; Poinar, 1990) and *H. bacteriophora*, respectively. These isolates were initially classified as *Xenorhabdus luminescens* (Thomas & Poinar, 1979). On the basis of phenotypic characters and DNA relatedness, the creation of a new genus, *Photorhabdus*, was proposed to accommodate the bacteria previously identified as *X. luminescens* (Boemare *et al.*, 1993); a single species, *Photorhabdus luminescens*, was described. However, several authors have shown that these organisms form a heterogeneous group on the basis of DNA–DNA hybridization (Akhurst *et al.*, 1996; Farmer *et al.*, 1989), 16S rDNA sequencing (Liu *et al.*, 1997; Rainey *et al.*, 1995; Szállás *et al.*, 1997) and PCR ribotyping (Brunel *et al.*, 1997). Recently, we re-examined the biodiversity among a collection of 92 *Photorhabdus* isolates from various regions of the world and our results, which were based on 16S rDNA polymorphism, led us to delineate twelve 16S RFLP types (Fischer-Le Saux *et al.*, 1998). Because of the relatively high level of genotypic diversity, we questioned the taxonomic status of these 16S rDNA groups, particularly whether they belong to the same or different species.

A polyphasic approach, which is the most reliable method for distinguishing species, was applied to

representative strains of the different groups inferred previously by 16S rDNA PCR-RFLP (Fischer-Le Saux *et al.*, 1998). We applied the following methods: DNA relatedness associated with ΔT_m measurements, phenotypic tests including morphological, biochemical and physiological characters, and complete 16S rRNA gene sequencing. On the basis of our results, we propose that two new species, *Photorhabdus temperata* and *Photorhabdus asymbiotica*, be created, the former including one subspecies, *P. temperata* subsp. *temperata* subsp. nov. We also propose that three subspecies of *P. luminescens* should be recognized: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov. and *P. luminescens* subsp. *laumondii* subsp. nov.

METHODS

Bacterial strains. The sources of bacterial isolates are listed in Table 1. They are representatives of the different 16S rDNA genotypes defined by PCR-RFLP (Fischer-Le Saux *et al.*, 1998). Except for the clinical strains, they were isolated from *Heterorhabditis* nematodes. The systematic status of the genus *Heterorhabditis* is still uncertain, because they are morphologically conserved and positive identification requires DNA fingerprinting and cross-breeding techniques. This is particularly true for the nematode strain Brecon (bacterial symbiont Hb^T) isolated in Australia, strain HP88 isolated in Utah (USA) and strain NC1 (bacterial symbiont C1) isolated in North Carolina (USA). These three nematode strains are generally considered today to belong to *H. bacteriophora* (Hominick *et al.*, 1996), but have some differences. Strain HP88 probably belongs to a species distinct from *H. bacteriophora* (Dix *et al.*, 1992), for which the type strain is Brecon. Satellite DNA from HP88 hybridizes at 100% with many other strains, but only at 50% with the type strain Brecon (Grenier *et al.*, 1996). Strain NC1 (= *Chromonema heliothidis*; Khan *et al.*, 1976), given the synonym *H. bacteriophora* (Poinar, 1976) on the basis of ecological arguments (Khan *et al.*, 1976), is probably also different from the Brecon strain. Therefore, we have included the strain designation after the species name to distinguish between the *H. bacteriophora* designations.

DNA extraction. Total DNA from pure cultures was extracted according to Brenner *et al.* (1982) for DNA–DNA hybridizations, amplification and sequencing.

DNA hybridizations and thermal stability of duplexes. Total DNA from strains Hb^T, XINach^T, 3265-86^T, FRG04^T and TT01^T was labelled *in vitro* by nick-translation using ³H-labelled nucleotides (Amersham). Levels of DNA relatedness were determined by using the S1 nuclease/TCA method (Grimont *et al.*, 1980) at the optimal temperature for DNA reassociation (65 °C) for 16 h. Each reaction was carried out in duplicate and means were taken. The 0% control hybridization was obtained from reassociation of the labelled DNA with herring sperm DNA and the 100% control was obtained by homologous hybridization. Heterologous hybridization values were normalized using 0 and 100%

Table 1. *Photorhabdus* strains used in this study

Strain names are given according to the usual nomenclature of the strains as described previously, where appropriate. New designations indicate a code for the country of isolation (DO, Dominican Republic; FRG, Guadeloupe; FRM, Martinique; JM, Jamaica; TT, Trinidad and Tobago) followed by a number. The 16S rDNA genotypes are defined in Fischer-Le Saux *et al.* (1998). The proposed nomenclature for each group and sub-group is given. Abbreviations: CIP, Collection de l'Institut Pasteur (Paris, France); NR, not reported.

Strain	Nematode host	Geographical origin (or reference)	Source†	16S rDNA genotype	Accession number
Group I (<i>Photorhabdus luminescens</i>)					
C8406	<i>Heterorhabditis</i> sp.*	China (Hainan island)	R. Akhurst ^a	11	NR
Sub-group IA (<i>Photorhabdus luminescens</i> subsp. <i>luminescens</i>)					
Hb [†] , HB1 (= ATCC 29999 [†])	<i>H. bacteriophora</i> Brecon [‡]	Australia (Victoria)	R. Akhurst ^a	10	X82248
Hm	<i>Heterorhabditis</i> sp.	USA (Georgia)	K. Neelson ^a	10	Z76742
Sub-group IB (<i>Photorhabdus luminescens</i> subsp. <i>akhurstii</i>)					
FRG04 [†] (= CIP 105564 [†])	<i>H. indica</i>	Guadeloupe	H. Mauléon ^b	12	AJ007359
P2M	<i>H. indica</i>	Cuba	E. Arteaga ^a	12	NR
DO03	<i>H. indica</i>	Dominican Republic	L. Garrido ^b	12	NR
DO04	<i>H. indica</i>	Dominican Republic	L. Garrido ^b	12	NR
DO10	<i>H. indica</i>	Dominican Republic	L. Garrido ^b	12	NR
PR16	<i>H. indica</i>	Puerto Rico	W. Figueroa ^b	12	NR
PR19	<i>H. indica</i>	Puerto Rico	W. Figueroa ^b	12	NR
FRM03	<i>H. indica</i>	Martinique	H. Mauléon ^b	12	NR
IS5	<i>H. ingica</i>	Israel	I. Glazer ^b	12	Z76745
JM12	<i>H. indica</i>	Jamaica	H. Mauléon ^b	27	NR
FRM05	<i>H. indica</i>	Martinique	H. Mauléon ^b	27	NR
FRG21	<i>H. indica</i>	Guadeloupe	H. Mauléon ^b	27	NR
D1	<i>H. indica</i>	Australia (Northern Territory)	R. Akhurst ^a	27	NR
Sub-group IC (<i>Photorhabdus luminescens</i> subsp. <i>laumondii</i>)					
HP88	<i>H. bacteriophora</i> HP88	USA (Utah)	R. Akhurst ^a	13	Z76743
TT01 [†] (= CIP 105565 [†])	<i>H. bacteriophora</i> HP88	Trinidad	H. Mauléon ^b	13	AJ007404
K80	<i>Heterorhabditis</i> sp.	Argentina	M. De Doucet ^b	13	NR
FRG01	<i>H. bacteriophora</i> HP88	Guadeloupe	H. Mauléon ^b	13	NR
PR02-A	<i>H. bacteriophora</i> HP88	Puerto Rico	W. Figueroa ^b	13	NR
Group II (<i>Photorhabdus temperata</i>)					
Meg	<i>H. megidis</i>	USA (Ohio)	R. Akhurst ^a	15	Z76750
C1 (= ATCC 29304)	<i>H. bacteriophora</i> NC1	USA (North Carolina)	R. Akhurst ^a	16	X82249
NZH3	<i>H. zealandica</i>	New Zealand	W. Wouts ^a	17	NR
Sub-group IIA (<i>Photorhabdus temperata</i> subsp. <i>temperata</i>)					
XINach [†] (= CIP 105563 [†])	<i>H. megidis</i> NTP	Russia	R. Akhurst ^a	14	AJ007405
HL81	<i>H. megidis</i> NTP	The Netherlands	P. Westerman ^a	14	NR
HW79	<i>H. megidis</i> NTP	The Netherlands	P. Westerman ^a	14	NR
lH211	<i>Heterorhabditis</i> sp.	Italy	K. Deseo ^a	14	NR
Group III (<i>Photorhabdus asymbiotica</i>)					
1216-79 (= ATCC 43948)	Clinical specimen	Farmer <i>et al.</i> (1989)	R. Akhurst ^a	29	NR
2407-88 (= ATCC 43952)	Clinical specimen	Farmer <i>et al.</i> (1989)	R. Akhurst ^a	29	Z76753
2617-87 (= ATCC 43951)	Clinical specimen	Farmer <i>et al.</i> (1989)	R. Akhurst ^a	29	Z76754
3105-77 (= ATCC 43949)	Clinical specimen	Farmer <i>et al.</i> (1989)	R. Akhurst ^a	29	Z76752
3265-86 [†] (= ATCC 49950 [†])	Clinical specimen	Farmer <i>et al.</i> (1989)	R. Akhurst ^a	29	Z76755

* Species of *Heterorhabditis* not yet identified.

† The species status of *H. bacteriophora* is uncertain and three subgroups have been recognized by various researchers (see Methods).

‡ Cultures were subcultures obtained from the indicated source (a) or were isolated in our laboratory from a nematode provided by the indicated source (b).

controls, to determine the percentage of relative DNA binding between two bacterial strains.

The thermal stability of reassociated DNAs was estimated by determining T_m , the temperature at which 50% of the DNA is single stranded. The T_m was determined by using a method described previously (Fernandez *et al.*, 1989), with slight modifications. The temperature of the hybridization mixture was increased from 65 to 95 °C in 5 °C increments. The hybridization mixture was not diluted with distilled water (final NaCl concentration, 0.42 M) after DNA hybridization was completed. The divergence between DNAs was estimated by calculating the ΔT_m value (difference between T_m of the homologous and heterologous duplexes).

Nucleotide sequencing of 16S rRNA genes. The 16S rRNA

genes of strains FRG04[†], TT01[†] and XINach[†] were amplified by PCR under conditions described previously (Brunel *et al.*, 1997). The following prokaryote-specific primers were used: 5'-GAAGAGTTTGATCATGGCTC-3' and 5'-AAGGAGGTGATCCAGCCGCA-3'. Amplified products were purified from an agarose gel with a Nucleotrap Extraction kit (Macherey-Nagel). Sequencing was performed by Act Gene-Euro Sequence Genes Services on an ABI377 sequencer using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase, FS (Perkin-Elmer). Six primers, 16S-F1 (5'-AGCCATGCCGCGTGTATG-3'), 16S-F2 (5'-GGGAGCAAACAGGATTAGAT-3'), 16S-F3 (5'-GAAATGTTGGGTAAAGTCCC-3'), 16S-R1 (5'-TGCAATATTCCCTCCACTGC-3'), 16S-R2 (5'-TTCCTCCACATCTCTACG-3') and 16S-R3

(5'-CGCTCGTTGCGGGACTTA-3'), as well as the two PCR primers, were used to sequence both strands of the 16S rDNA.

DNA sequence analysis. GenBank was searched for related sequences using the algorithm BLAST (Altschul *et al.*, 1997) and related sequences of *Photorhabdus* strains were included in the subsequent analyses. Sequences were aligned using CLUSTAL-X (Thompson *et al.*, 1997). Matrix pairwise comparisons of nucleic acid sequences were corrected for multiple base substitutions by the two-parameter method of Kimura (1980). Pairwise evolutionary distances were computed using the Poisson correction for multiple substitutions (Nei, 1987). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and parsimony (Kluge & Farris, 1969) methods. A bootstrap confidence analysis was performed on 1000 replicates to determine the reliability of the distance-tree topologies obtained (Felsenstein, 1985). Graphic representation of the resulting trees was made using the NJPLOT and PHYLO_WIN software (Perrière & Gouy, 1996).

Phenotypic characterization. All the cultures were duplicated weekly on MacConkey agar or on nutrient agar supplemented with 0.004% (w/v) triphenyltetrazolium chloride and 0.0025% (w/v) bromothymol blue (NBTA medium) and controlled to prevent a mixture of phase I and phase II variants (Boemare & Akhurst, 1988). Phase I and phase II isolates were examined independently and positive responses were combined to be assigned to the original strain. In most of the cases, while phase I variants responded positively, phase II variants were negative, but in all cases it was the positive response that was scored for the corresponding strain. Phenotypic characters were scored at 28 °C, using the same methods as Boemare & Akhurst (1988), except for annular haemolysis, which was tested at 25 °C (Akhurst *et al.*, 1996; Farmer *et al.*, 1989), and the determination of maximum growth temperatures. Acidification of carbohydrates was determined on API 50 CH strips with API 50 CHE medium (bioMérieux), using phenol red as the pH indicator. Utilization of carbohydrates and of organic and amino acids was tested either on API 50 CH, 50 AO and 50 AA strips with API LRA medium, or on Biotype 100 by using the minimal medium II (bioMérieux). We checked that test results were identical whichever commercial preparations was used, and only the results obtained from the 76 substrates shared by both strips were scored for further analysis.

The maximum temperature at which each strain grew was determined by inoculating cultures in nutrient broth maintained at a calibrated temperature in a water bath (Julabo), electronically adjusted to within ± 0.1 °C, and the temperature was monitored with a mercury thermometer (graduations of 0.05 °C). Temperatures ranging from 30 to 42 °C were tested in 1 °C increments. To avoid any pre-selection from the original strains, the inocula for these tests were taken from cultures maintained at 28 °C.

RESULTS

DNA-DNA hybridizations

The unstandardized reassociation values for homoduplexes (100% control) obtained by the S1 nuclease

method ranged from 71 to 97%. The levels of reassociation in negative (0%) controls ranged from 2 to 11%. The T_m values for homoduplexes in 0.42 M NaCl ranged from 88.5 to 92.0 °C. The percentage DNA relatedness between strains and the ΔT_m values are shown in Table 2.

The 23 strains studied fell into three genomic groups (I–III). Within genomic group I, strains had at least 48% DNA relatedness to Hb^T, FRG04^T and TT01^T, with ΔT_m values of 0.5–7.7 °C. On the other hand, strains of genomic group I had low DNA binding (25–42% by the S1 nuclease method) associated with high divergence values (ΔT_m greater than 8.9 °C) with strains outside this group.

Genomic group II encompassed six strains with 51–100% DNA relatedness to strain XINach^T with low levels of divergence (ΔT_m less than 5.7 °C), and yielded low DNA binding (less than 42%) and high ΔT_m values (greater than 8.7 °C) with all the other strains.

Genomic group III contained the two medical strains, 3265-86^T and 3105-77. They were closely related, since they showed high DNA relatedness values (greater than 92%) with very low divergence (ΔT_m 0.5 °C). They were clearly separated from members of the other two groups by low DNA reassociation (28–33%) and high ΔT_m values (8.7–10.7 °C).

Varied DNA reassociation values were observed within genomic group I, and three genomic sub-groups were identified (IA–IC). Sub-group IA contained the type strain of *P. luminescens*, Hb^T, and strain Hm, which were 100% related, and both belonged to 16S rDNA RFLP genotype 10. Sub-group IB contained five strains, which had a high level of relatedness to strain FRG04^T (more than 83%) with very low ΔT_m values (less than 1 °C). All five strains were symbionts of the same nematode species, *Heterorhabditis indica* (Table 1). Sub-group IC included four strains with a high level of relatedness to strain TT01^T (more than 83%) and low ΔT_m values (less than 1.3 °C). Like strain TT01^T, they all belonged to 16S rDNA RFLP genotype 13 and were all symbionts of the HP88 sub-group of *H. bacteriophora*. Strain C8406 of genomic group I remained unclassified within these sub-groups, since it did not hybridize at a high level with Hb^T, FRG04^T or TT01^T.

Within genomic group II, one genomic sub-group (IIA) was distinguished and contained two strains, XINach^T and HL81, with 100% DNA relatedness and a ΔT_m of 0.3 °C. These two strains belonged to genotype 14 and were symbionts of the northern and temperate Palaearctic (NTP) sub-group of *Heterorhabditis megidis* (see Table 1). Strain HW79, also of genotype 14 and from the same *H. megidis* NTP sub-group, probably also belonged to this sub-group, but the lack of DNA pairing values prevented confirmation of this. Strains Meg, C1 and NZH3 were less related to strain XINach^T, with 51–54% DNA related-

Table 2. Levels of DNA relatedness and ΔT_m between *Photorhabdus* strainsStrain names are followed by PCR-RFLP 16S rDNA genotypes in square brackets (Fischer-Le Saux *et al.*, 1998). Numbers in parentheses are ΔT_m (°C).

Source of unlabelled DNA			Source of labelled DNA								
Group	Strain	Hb ^T *	Hb ^T †	FRG04 ^T †	TT01 ^T †	XINach ^T †	C1*	Meg*	NZH3*	3265-86 ^T †	1216-79*
I	Subgroup IA										
	Hb ^T [10]	100	100	65 (7.7)	48 (7.3)	31	51	57	45	32	
	Hm [10]	109							57		
	Subgroup IB										
	FRG04 ^T [12]		54 (6.2)	100	49 (7.6)	40				31	
	P2M [12]			92 (0.5)	54 (6.6)					30 (10.7)	
	DO04 [12]		53 (6.5)	95	52 (6.5)					31 (10.3)	
	JM12 [27]		57 (5.9)	83 (1.0)	48 (5.7)						
	FRM05 [27]			99	49 (6.3)	33				33 (9.7)	
	Subgroup IC										
	TT01 ^T [13]		55 (6.3)	72 (5.5)	100	30 (10.8)				31	
	HP88 [13]	71		60 (6.7)	83 (0.8)	31	44	51	51	28	
	K80 [13]	57		70 (6.0)	90 (1.3)		36	56	54	31	
II	FRG01 [13]		51 (6.4)	62 (6.9)	90					31 (8.9)	
	C8406 [11]	77	54 (6.5)	67 (6.0)	62 (4.6)	35	37	59	48	28	
	Subgroup IIA										
	X1Nach ^T [14]	48	29 (9.4)		33 (9.4)	100	65	73	51	32 (8.7)	54
	HL81 [14]	52		38 (9.1)	29	104 (0.3)	60	78	75	31	
	HW79 [14]	44					73	76	65		
	Meg [15]	32	36 (10.2)	41 (10.9)	28	54 (4.9)	91	100	58	32 (9.8)	53
	C1 [16]	32				51 (4.5)	100	75	66	28	
	NZH3 [17]	47	34 (9.3)	42 (9.8)	31	52 (5.7)	64	67	100	30	
	3265-86 ^T [29]		31 (9.4)	35 (12.9)	26	28				100	100
	3105-77 [29]				25		29	55		92 (0.5)	107
	1216-79 [29]	44					50	60	56	100	100
	2407-88 [29]							50		103	103
2617-87 [29]	46					41	41	41	100	100	

* DNA relatedness data obtained by hydroxyapatite (Akhurst *et al.*, 1996).

† DNA relatedness data obtained by S1 nuclease/TCA method (this study).

Fig. 1. Phylogenetic tree of *Photothabdus* species 16S rRNA obtained by the neighbour-joining method (Saitou & Nei, 1987) using a bootstrap approach (Felsenstein, 1985) to determine the reliability of the topology obtained (numbers given above the nodes). Those clusters also obtained by parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) analyses are indicated by P and L below the nodes, respectively. Accession numbers are given in front of strain names. The bar indicates a distance of 0.002 substitutions per site.

ness and ΔT_m of 4.5–5.7 °C. Although they belonged to group II, they were excluded from sub-group IIA.

Sequencing and analysis of 16S rDNA

The primers used for PCR amplification of 16S rDNA of strains FRG04^T, TT01^T and XINach^T yielded approximately 1550 bp amplicons. Nearly full-length double-strand sequences of these amplicons were obtained. Using the algorithm BLAST, *Photothabdus* 16S rRNA genes were confirmed as the most closely related sequences. Sequences determined in this study were compared to the complete 16S rDNA sequences of *Photothabdus* isolates available from databases for which the nematode host species was identified. The phylogenetic tree obtained by the neighbour-joining method is shown in Fig. 1.

Strains from genomic group I (Hb^T, Hm, HP88, TT01^T and FRG04^T) clustered together and three sub-clusters corresponding to the three genomic sub-groups, IA, IB and IC, were clearly distinguished. They also clustered together with another *Photothabdus* strain, IS5, the sequence of which was available (Szlls *et al.*, 1997). All sub-clusters within group I were supported by bootstrap results of 100% and by parsimony and maximum-likelihood analyses. Each genomic sub-group formed a relatively tight cluster with similarity

coefficients ≥ 0.992 (12 nucleotide substitutions observed). Genomic sub-group IA, which contained the type strain Hb^T, branched deeply and represented the nearest phylogenetic neighbour of the medical strains.

Strains of genomic group II (Meg, C1 and XINach^T) clustered together with two other *Photothabdus* strains, HSH2 and PE87.3, for which sequences were available. This cluster had a bootstrap confidence value of 97% and was also found by maximum-likelihood and parsimony analyses. Two sub-clusters were clearly delineated: the first included strains Meg and C1, which were relatively close, with 13 nucleotide differences observed. The second sub-cluster included strain XINach^T and the two strains HSH2 and PE87.3, which were closely related to XINach^T with only seven and six nucleotide differences, respectively. Strains HSH2 and PE87.3 were isolated from the NTP sub-group of *H. megidis*, as was XINach^T, which is a component of the genomic sub-group IIA.

The four medical strains for which sequences are available formed a very tight cluster, corresponding to group III, with a similarity coefficient of 0.999. This cluster was supported by a bootstrap value of 100% and by maximum-likelihood and parsimony analyses.

Phenotypic data

Phenotypic tests were performed with 33 representative strains belonging to *Photothabdus* groups (21 strains from group I, seven from group II and five from group III) and sub-groups (Table 3). All the strains studied were Gram-negative, rod-shaped bacteria, motile with peritrichous flagella. All phase I variants adsorbed dyes from MacConkey agar and NBTA medium and produced antimicrobial activity against *Micrococcus luteus*. All colonies were pigmented, but phase I and phase II variants were pigmented differently. All the strains were catalase- and bioluminescence-positive. The following seven tests were also positive for all the strains tested: gelatin (Kohn's) hydrolysis, hydrolysis of Tweens 20, 60, 80 and 85, and haemolysis, tested on horse-blood agar. Nine classical tests were recorded as negative for all strains: nitrate reduction to nitrite, oxidase activity, Voges–Proskauer reaction, hydrogen sulfide production, β -galactosidase activity on *o*-nitrophenyl β -D-galactopyranoside and *p*-nitrophenyl β -D-galactopyranoside and activities of lysine and ornithine decarboxylases and arginine dihydrolase. Clinical strains, considered as phase II, did not adsorb dyes and produced antibiotic activity weakly when positive, and most of them produced a yellow pigment.

A total of 49 carbon sources were tested for acid production and 76 for assimilation. None of the strains used or fermented the following compounds: adonitol, L-arabinose, D- and L-arabitol, D-cellobiose, dulcitol, erythritol, D-galactose, β -gentiobiose, 5-keto-D-glucuronate, α -lactose, D-lyxose, D-melezitose, α -D-melibiose, D-raffinose, L-sorbose, D-tagatose, D-turanose and xylitol. No strain produced acid from D-arabinose,

Table 3. Characteristics that differentiate between *Photorhabdus* groups

All tests were done at 28 ± 1 °C unless otherwise noted. Results of tests are indicated as follows: +, more than 90 % of strains positive; [+], 76–89 % positive; d, 26–75 % positive; [–], 11–25 % positive; –, fewer than 10 % positive. The superscript w (e.g. [+]^w) indicates a weak reaction. NA, Not applicable. Numbering of genotypes is according to Fischer-Le Saux *et al.* (1998). Results for the type strains are given in parentheses.

	Group I	Strain C8406	Sub-group IA	Sub-group IB	Sub-group IC	Group II	Strain Meg	Strain C1	Strain NZH3	Sub-group IIA	Group III
Proposed nomenclature	<i>P. luminescens</i>	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>luminescens</i>	<i>P. luminescens</i> subsp. <i>akhurstii</i>	<i>P. luminescens</i> subsp. <i>laumondii</i>	<i>P. temperata</i>	<i>P. temperata</i>	<i>P. temperata</i>	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>temperata</i>	<i>P. asymbiotica</i>
Isolated from	<i>Heterorhabditis</i> spp.	<i>Heterorhabditis</i> sp.	<i>H. bacteriophora</i> Brecon	<i>H. indica</i>	<i>H. bacteriophora</i> HP88	<i>Heterorhabditis</i> spp.	<i>H. megidis</i>	<i>H. bacteriophora</i> NC1	<i>H. zealandica</i>	<i>H. megidis</i> NTP	Human blood and/or wounds
Type strain	ATCC 29999 ^T	NA	ATCC 29999 ^T	CIP 105564 ^T	CIP 105565 ^T	CIP 105563 ^T	NA	ATCC 29304 ^T	NA	CIP 105563 ^T	ATCC 43950 ^T
Number of strains tested	21	1	2	13	5	7	1	1	1	4	5
PCR-RFLP 16S rDNA genotype	10–13, 27 (10)	11	10 (10)	12, 27 (12)	13 (13)	14–17 (14)	15	16	17	14 (14)	29 (29)
Maximum growth temp. (°C)	35–39 (38)	38	38–39 (38)	38–39 (38)	35–36 (36)	33–35 (34)	34	35	33	34 (34)	37–38 (38)
Indole	+ (+ ^w)	+	+ ^w (+ ^w)	d (+)	+ (+)	[–] (–)	–	–	+	– (–)	– (–)
DNase	[–] (–)	–	– (–)	– (–)	+ (+)	+ (+)	+	+	+	+ (+)	– (–)
Urease (Christensen's)	d (–)	–	– (–)	d (+ ^w)	[+] ^w (+ ^w)	d (–)	+	+	–	[–] (–)	+ (+)
Aesculin hydrolysis	+ (+)	–	+ (+)	+ (+ ^w)	+ (+)	[+] ^w (+)	+	+	+	d (+)	+ (+)
Tryptophan deaminase	– (–)	–	– (–)	– (–)	d (–)	[+] ^w (–)	+	+	+	d (–)	– (–)
Simmons' citrate	d (+ ^w)	–	+ (+ ^w)	d (+ ^w)	d (–)	d (+ ^w)	–	–	+	d (+ ^w)	+ ^w (+ ^w)*
Annular haemolysis on:											
Sheep-blood agar	d (+)	–	+ (+)	+ (+)	[–] (–)	d (+ ^w)	+	–	+	+ ^w (+ ^w)	+ (+)
Horse-blood agar	d (+)	–	+ (+)	d (–)	– (–)	[+] ^w (+)	+	+	+	+ (+)	+ (+)
Production of acid from:											
Mannitol	d (+ ^w)	–	d ^w (+ ^w)	+ (+ ^w)	– (–)	[–] (–)	–	–	+	– (–)	– (–)
Trehalose	[+] ^w (+ ^w)	–	+ ^w (+ ^w)	[+] ^w (+ ^w)	[+] ^w (–)	d (+)	–	+ ^w	+	+ (+)	[+] ^w (+)
Utilization of:											
L-Fucose	d ^w (+ ^w)	–	d (+ ^w)	[+] ^w (+ ^w)	– (–)	d (+)	–	–	–	+ (+)	– (–)
DL-Glycerate	– (+)	–	d (+)	– (–)	– (–)	+ (+)	+ ^w	+	+	+ (+)	d (+)
L-(–)-Histidine	d (+)	+	+ (+)	d (–)	[+] ^w (+ ^w)	[+] ^w (+)	–	+	+	+ (+)	d (+)
myo-Inositol	+ (+)	–	+ (+)	+ (+)	+ (+)	[+] ^w (–)	+	+	+	d (–)	d (+)
DL-Lactate	d ^w (–)	–	– (–)	d (+ ^w)	– (–)	– (–)	–	–	–	– (–)	– (–)
D-Mannitol	d (+)	–	+ (+)	+ (+)	– (–)	[–] (–)	–	–	+	– (–)	– (–)

* Weakly positive on Simmons' citrate but positive on Christensen's citrate (Farmer *et al.*, 1989).

arbutin, D-fucose, 2-ketogluconate, methyl D-glucoside, glycogen, inulin, methyl D-mannoside, rhamnose, sucrose, sorbitol, starch, D- or L-xylose or β -methyl D-xyloside. Most of the strains did not ferment amygdalin or salicin. None of the strains tested were able to assimilate the following compounds: benzoate, *m*- and *p*-hydroxybenzoate, betaine, 3-hydroxybutyrate, ethanolamine, 1-*O*-methyl α -D-glucopyranoside, glutarate, 2-oxoglutarate, histamine, itaconate, phenylacetate, putrescine, L-, D- and *meso*-tartrate, trigonelline, tryptamine and L-tryptophan. Very few strains grew with malonate, L-rhamnose, sucrose or D-sorbitol as sole sources of carbon. All strains produced acid from glucose without gas production. Most of the strains produced acid weakly from fructose, *N*-acetylglucosamine, glycerol, maltose, mannose and ribose. The following six compounds were always assimilated: fumarate, *N*-acetylglucosamine, glucose, L-glutamate, mannose and D-ribose. More than 90 % of the strains used L-alanine, D-fructose, D-gluconate, D-glucos-

amine, glycerol, maltose, L-proline, L-serine, succinate and L-tyrosine as sole sources of carbon.

The results of tests that can be used to distinguish between the *Photorhabdus* groups are listed in Table 3. Thus, members of group I were distinguished by their ability to produce indole. Members of group II exhibited DNase activity and assimilated DL-glycerate, while the majority of members of groups I and III did not. In contrast to groups I and II, members of group III never assimilated L-fucose, but were always positive for Christensen's urease and Simmons' citrate reactions. Some classical phenotypic tests were also useful to characterize sub-groups. Within group I, members of sub-group IC had DNase activity while members of sub-groups IA and IB were always negative. In contrast, strains of sub-group IC did not use or produce acid from L-fucose or D-mannitol, whereas numerous members of sub-groups IA and IB did. Some strains of sub-group IA were able to grow

Fig. 2. Interpretation of the DNA binding ratio data showing the levels of relatedness of *Photorhabdus* strains. Three clusters of DNA reassociation values were delineated: (i) more than 80% relatedness associated with $\Delta T_m < 1.5^\circ\text{C}$, which corresponds to the relatedness between members of the same subspecies (filled boxes); (ii) 50–70% relatedness with $\Delta T_m 4.5\text{--}7^\circ\text{C}$, which corresponds to the level of relatedness occurring among members of different subspecies within a species (shaded boxes); and (iii) less than 40% relatedness with ΔT_m higher than 9°C , which corresponds to reassociation values between members of different species (unfilled boxes). Numbers of 16S rRNA genotypes according to Fischer-Le Saux *et al.* (1998) are indicated in parentheses after the strain names.

with DL-lactate as the sole source of carbon. In contrast, members of the other sub-groups were unable to do so.

DISCUSSION

Recently, we described high genetic heterogeneity among strains belonging to the genus *Photorhabdus* and 12 ribosomal types were recognized by analysis of restriction patterns obtained after amplification of the 16S rDNA (Fischer-Le Saux *et al.*, 1998). In order to define the taxonomic status of these bacteria, we studied some representative strains among the well-represented 16S rDNA genotypes by DNA–DNA hybridization, which is the key method for delineation of bacterial species (Wayne *et al.*, 1987). A species is defined as a group of strains sharing approximately 70% or greater DNA–DNA relatedness with ΔT_m of 5°C or less. However, bacterial taxonomists recognize that these values are indicative, rather than absolute

(Vandamme *et al.*, 1996). On the basis of data obtained using the S1 nuclease method, Grimont (1988) made a more robust definition by stating that strains showing 80% reassociation with ΔT_m below 5°C belonged to one genomic species and that strains showing less than 60% reassociation and more than 7°C divergence did not belong to the same species. For values between 60 and 80% DNA relatedness or between 5 and 7°C divergence, strains should be studied carefully to delineate species and subspecies.

According to these rules (Vandamme *et al.*, 1996; Wayne *et al.*, 1987), we have defined three genomic groups that exhibited between them DNA–DNA hybridization values lower than 42% with ΔT_m greater than 8.7°C (as summarized in Fig. 2). Therefore, these three groups do not belong to the same species. In order to compare our data with previous results, DNA–DNA reassociation data obtained from the hydroxyapatite (HA) method (Akhurst *et al.*, 1996) were also included in Table 2. As reported before

(Grimont *et al.*, 1980), DNA reassociation values obtained with the S1 nuclease method are lower than those obtained with the HA method. The delineation of group II is also corroborated by the HA method (Akhurst *et al.*, 1996), with DNA relatedness ranging from 51 to 91 % within strains of group II and from 29 to 60 % when compared with strains of the other genomic groups. Genomic group III corresponded to a related tight genomic group (more than 92 % DNA relatedness with ΔT_m less than 0.5 °C, and between 100 and 107 % according to Akhurst *et al.*, 1996), whereas groups I and II were less homogeneous. Sub-groups were defined by a high DNA relatedness and a low ΔT_m (more than 83 % with $\Delta T_m < 1.3$ °C) (Fig. 2). Within genomic species, sub-groups showed between them about 50–70 % DNA reassociation with ΔT_m between 4.5 and 7 °C. Some values were slightly outside these limits, but if we consider the standard errors of the data as estimated by Grimont (1988) and Sneath (1989) they were not significantly different; in addition, reciprocal hybridization values were in agreement (< 7 °C). Moreover, the phylogenetic trees inferred from the complete 16S rDNA sequence analysis (neighbour-joining, parsimony and maximum-likelihood methods) delineate the same clusters as the DNA–DNA hybridizations. Members of genomic groups II and III clustered with high bootstrap confidence values, as did the members of sub-groups IA, IB and IC. In contrast, the clustering of these sub-groups within group I was less defined, with bootstrap values of 65 and 68 %. This must be due to the greater 16S rDNA divergence found in group I (up to 3.5 %). Therefore, on the basis of 16S rDNA similarities, genomic group I appears to be more divergent. Nevertheless, it forms one hybridization group (more than 50 % DNA relatedness, $\Delta T_m < 7$ °C).

By applying a polyphasic approach, combining 16S rDNA, DNA–DNA hybridization and phenotypic data, we were able to delineate species and subspecies in the genus *Photorhabdus*, whereas previous attempts had not been successful (Akhurst *et al.*, 1996; Rainey *et al.*, 1995). Previous RFLP data, collected from a large number of isolates, allowed us to choose several representatives of each 16S rDNA type for this study in order to constitute a more comprehensive sample and helped to define the appropriate strains to use. Thus, 16S rDNA RFLP genotype 10 (Fischer-Le Saux *et al.*, 1998) corresponds to sub-group IA of the present study, the two similar genotypes 12 and 27 to sub-group IB, genotype 13 to sub-group IC, genotype 14 to sub-group IIA and genotype 29 to group III (Table 3; Fig. 2). Furthermore, the determination of the ΔT_m , which was not available in our previous work (Akhurst *et al.*, 1996), was useful to remove remaining ambiguities generated by intermediate values ranging from 50 to 80 %. As more strains become available for each group, phenons with relevant taxonomic characters, if any, will be identified more easily and related to the taxa obtained by DNA–DNA hybridization and 16S rDNA sequencing. Some differential biochemical

characters that are relevant for use in classification are provided in the taxon descriptions given below.

Within the genus *Photorhabdus*, the maximum temperature at which growth occurs appears to be a relevant taxonomic character defining a critical value for each sub-group (Table 3). It implies essential physiological adaptations in the enzymic machinery of the bacteria and must therefore be considered as important for defining taxa. Moreover, if we examine the ecology of the corresponding strains, it is noticeable that the maximum growth temperature and the host species origin are correlated. Symbionts growing at temperatures of up to 35–39 °C are harboured by *H. bacteriophora* (Brecon, HP88) and *H. indica*, which occur in warm regions. Those which only grow at up to 35 °C are harboured by *H. megidis*, including its NTP strains, and *H. bacteriophora* (NC1); all of these nematodes live in temperate climates. Thus, temperature tolerance appears to be an important property reflecting a long-term adaptation to different climatic conditions.

Photorhabdus is revealed as a heterogeneous genus, within which further sub-groups should be defined in the future when more strains become available: for example, 16S rDNA genotype 11 in group I and genotypes 15–17 in group II could constitute at least two and probably more new subspecies. Some distinctive phenotypic characters can already be distinguished for some of these strains; for instance, strain NZH3 in group II is indole-positive and uses mannitol.

Unfortunately, it appears that the type strain of the genus *Photorhabdus* and of the species *P. luminescens* (strain Hb^T) is not a good representative of the *Photorhabdus* isolates, because, as numerous new isolates are typed, none have been found to belong to this taxon. So far, strain Hm remains the only one closely related to the type strain Hb^T.

Conclusions

Due to their low genomic relatedness (< 42 %) and high divergence ($\Delta T_m > 8.7$ °C), their deep phylogenetic relationship and their differential phenotypic characters, we propose the existence of three species among the *Photorhabdus* isolates (as summarized in Fig. 2). These are: (i) *P. luminescens*, containing the type strain and the symbiotic strains of nematodes that are ubiquitous and/or native to warm regions (maximum growth temperature 35–39 °C), (ii) *P. temperata* sp. nov., containing exclusively the symbiotic strains of nematodes native to temperate regions (maximum growth temperature 33–35 °C), and (iii) *P. asymbiotica* sp. nov., originating from clinical samples. Strains isolated from human patients can be clearly distinguished from the nematode symbionts.

P. luminescens and *P. temperata* are heterogeneous genomic groups, but because their DNA–DNA hybridization values were relatively high with ΔT_m lower

than 7 °C and because each of them constituted a coherent phenotypic group based on its maximum growth temperature, we propose to delineate only two species. However, since sub-groups of strains within these two species shared very high DNA–DNA hybridization values and ΔT_m lower than 1.5 °C and were separated by stable 16S rDNA branching, we propose three subspecies within *P. luminescens*: *P. luminescens* subsp. *luminescens* subsp. nov., containing the type strain and another strain associated with nematodes from the Brecon sub-group of *H. bacteriophora*; *P. luminescens* subsp. *akhurstii* subsp. nov., containing strains associated with *H. indica*; and *P. luminescens* subsp. *laumondii* subsp. nov., containing strains associated with the HP88 sub-group of *H. bacteriophora*. Similarly, we propose a subspecies within *P. temperata*, containing strains associated with the NTP sub-group of *H. megidis* and named *P. temperata* subsp. *temperata* subsp. nov. since it contains the type strain. Consequently, there is a close correspondence between the taxonomic grouping of the bacteria and that of their nematode associates.

According to the rule 65 (2) of the Code of nomenclature, generic and subgeneric names that are modern compounds of two or more Latin or Greek words take the gender in the original language of the last component of the compound word. Consequently, *Photorhabdus* (Pho.to.rhab'dus. Gr. n. *phos* light; Gr. fem. n. *rhabdos* rod) becomes a feminine word in modern Latin. This explains the proposed feminine names of species and subspecies, as detailed below.

Emended description of *Photorhabdus luminescens* (Thomas and Poinar, 1979) Boemare, Akhurst and Mourant 1993

Photorhabdus luminescens (lu.mi.nes'cens. M.L. pres. part. *luminescens* luminescing; for its bioluminescence).

Cells are large rods (2–6 × 0.5–1.4 µm). Occurs as two phase variants. Bioluminescent, with bioluminescence more than 100-fold greater in phase I. Maximum temperature for growth in nutrient broth is 35–39 °C. Indole-positive. Most strains produce acid weakly from fructose, *N*-acetylglucosamine, glucose, glycerol, maltose, mannose, ribose and trehalose. Some strains acidify mannitol. Proteinaceous inclusions in phase I cell protoplasm, produced poorly in phase II. The natural habitat is the intestinal lumen of entomopathogenic nematodes of the species *H. bacteriophora* (Brecon and HP88 sub-groups) and *H. indica*. This species is divided into three subspecies.

The type strain (Hb^T) is held by the American Type Culture Collection under accession number ATCC 29999^T. The EMBL accession number of the 16S rRNA gene sequence of strain DSM 3368 (paratype of ATCC 29999^T) (Rainey *et al.*, 1995) is X82248.

Description of *Photorhabdus luminescens* subsp. *luminescens* subsp. nov.

Photorhabdus luminescens subsp. *luminescens* (lu.mi.nes'cens. M.L. pres. part. *luminescens* luminescing; for its bioluminescence).

Maximum temperature for growth in nutrient broth is 38–39 °C. Aesculin hydrolysis-positive and weakly indole-positive. Negative for DNase, tryptophan deaminase and urease. Annular haemolysis of sheep- and horse-blood agars. Does not use DL-lactate as sole source of carbon. Mannitol used as sole source of carbon and energy. Symbiotically associated with nematodes from the Brecon sub-group of *H. bacteriophora*, the type species of the genus *Heterorhabditis* (Poinar, 1976).

The type strain, Hb^T, is held in the American Type Culture Collection under accession number ATCC 29999^T. The EMBL accession number of the 16S rRNA gene sequence of the strain DSM 3368 (paratype of ATCC 29999^T) (Rainey *et al.*, 1995) is X82248.

Description of *Photorhabdus luminescens* subsp. *akhurstii* subsp. nov.

Photorhabdus luminescens subsp. *akhurstii* (ak.hurs'ti.i. M.L. gen. n. *akhurstii* of Akhurst, referring to R. Akhurst, a major contributor to the bacteriological symbionts of entomopathogenic nematodes).

Maximum temperature for growth in nutrient broth is 38–39 °C. Aesculin hydrolysis-positive. Negative for tryptophan deaminase and DNase. Urease and indole variable. Annular haemolysis observed on sheep-blood agar and, in some strains, on horse-blood agar. Utilization of DL-lactate as sole source of carbon variable; weak when positive. Mannitol used and acidified. DL-Glycerate utilization is negative. Symbiotically associated with the nematode *H. indica* isolated in warm regions, the first strain (D1) was isolated from Australia (Darwin, Northern Territory) by R. Akhurst.

The type strain, FRG04^T, is held in the Collection of the Institut Pasteur under accession number CIP 105564^T. The EMBL accession number of the 16S rRNA gene sequence of the type strain is AJ007359.

Description of *Photorhabdus luminescens* subsp. *laumondii* subsp. nov.

Photorhabdus luminescens subsp. *laumondii* (lau.mon'di.i. M.L. gen. n. *laumondii* of Laumond, referring to C. Laumond, a major contributor to the use of entomopathogenic nematode/bacterial complexes for insect pest control).

Maximum temperature for growth in nutrient broth is 35–36 °C. Positive for aesculin hydrolysis, indole and DNase. Tryptophan deaminase variable and mostly urease-positive. Total haemolysis on sheep- and horse-blood agars (the *Photorhabdus* annular reaction is

rare). Does not use L-fucose, DL-glycerate, DL-lactate or mannitol. Symbiotically associated with nematodes of the HP88 sub-group of *H. bacteriophora* isolated in South and North America, southern Europe and Australia, responding to the satellite DNA probe of the nematode strain HP88 (Grenier *et al.*, 1996) provided by the team of C. Laumond.

The type strain, TT01^T, is held in the Collection of the Institut Pasteur under accession number CIP 105565^T. The EMBL accession number of the 16S rRNA gene sequence of the type strain is AJ007404.

Description of *Photorhabdus temperata* sp. nov.

Photorhabdus temperata (tem.pe.ra'ta. L. fem. part. adj. *temperata* moderate, because this species grows at moderate temperatures).

Cells are large rods (2–6 × 0.5–1.4 µm). Occurs as two phase variants. Highly bioluminescent. Maximum temperature for growth in nutrient broth is 33–35 °C. DNase-positive. Most of the strains are indole-negative. Aesculin hydrolysis and tryptophan deaminase mostly positive. Urease variable. Acid produced from fructose, *N*-acetylglucosamine, glucose, mannose and ribose; weak acid production from glycerol and maltose. Proteinaceous inclusions in phase I cell protoplasm, produced poorly in phase II. Annular haemolysis of sheep- and horse-blood agars often occurs. Uses DL-glycerate and does not use DL-lactate as sole sources of carbon. Mannitol is not used by most strains. The natural habitat is the intestinal lumen of entomopathogenic nematodes belonging to *H. megidis*, the NC1 sub-group of *H. bacteriophora* and *H. zealandica*.

The type strain, XINach^T, is held in the Collection of the Institut Pasteur under accession number CIP 105563^T. The EMBL accession number of the 16S rRNA gene sequence of the type strain is AJ007405.

Description of *Photorhabdus temperata* subsp. *temperata* subsp. nov.

Photorhabdus temperata subsp. *temperata* (tem.pe.ra'ta. L. fem. part. adj. *temperata* moderate, because this species grows at moderate temperatures; so named because this subspecies contains the type strain of the species).

Cells are large rods (2–6 × 0.5–1.4 µm). Occurs as two phase variants, sometimes with several intermediate forms. Bioluminescent. Maximum temperature for growth in nutrient broth is 34 °C. Indole-negative, DNase-positive. Aesculin hydrolysis and tryptophan deaminase variable. Mostly urease-negative. Annular haemolysis on sheep- and horse-blood agars in most isolates. Uses DL-glycerate and L-fucose and does not use DL-lactate or mannitol as sole sources of carbon. The natural habitat is the intestinal lumen of entomopathogenic nematodes of the NTP sub-group of *H. megidis*.

The type strain, XINach^T, is held in the Collection of the Institut Pasteur under accession number CIP 105563^T. The EMBL accession number of the 16S rRNA gene sequence of the type strain is AJ007405.

Description of *Photorhabdus asymbiotica* sp. nov.

Photorhabdus asymbiotica (a.sym.bio'ti.ca. Gr. pref. *a* not; M.L. fem. adj. *symbiotica* living together; M.L. fem. adj. *asymbiotica* not symbiotic).

Cells are rods of 2–3 × 0.5–1.0 µm. Maximum temperature for growth in nutrient broth is 37–38 °C. Yellow or brown pigment. No phase I isolates have been detected and isolates do not absorb dyes, sometimes produce antibiotics weakly and are negative for lecithinase on egg-yolk agar. Positive for urease, aesculin hydrolysis and Christensen's citrate, but weakly positive on Simmons' citrate. Tryptophan deaminase-negative. Indole- and DNase-negative. Acid produced from fructose, *N*-acetylglucosamine, glucose, maltose, mannose and ribose, produced weakly from glycerol. Proteinaceous inclusions produced poorly. Tween 40 esterase variable. Annular haemolysis of sheep- and horse-blood agars. Does not use L-fucose, DL-lactate or mannitol. Natural habitat uncertain. All isolates obtained from human clinical specimens.

Strain 3265-86^T (= ATCC 43950^T) is designated as the type strain, as suggested previously by Farmer *et al.* (1989). The EMBL accession number of the 16S rRNA gene sequence of the type strain (Szállás *et al.*, 1997) is Z76755.

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