Phylogeny of *Photorhabdus* and *Xenorhabdus* based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: *X. vietnamensis* sp. nov., *P. luminescens* subsp. *caribbeanensis* subsp. nov., *P. luminescens* subsp. *hainanensis* subsp. nov., *P. temperata* subsp. *khanii* subsp. nov., *P. temperata* subsp. *tasmaniensis* subsp. nov., and the reclassification of *P. luminescens* subsp. *thracensis* as *P. temperata* subsp. *thracensis* comb. nov.

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We used the information from a set of concatenated sequences from four genes (*recA*, *gyrB*, *dnaN* and *gltX*) to investigate the phylogeny of the genera *Photorhabdus* and *Xenorhabdus* (entomopathogenic bacteria associated with nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively). The robustness of the phylogenetic tree obtained by this multigene approach was significantly better than that of the tree obtained by a single gene approach. The comparison of the topologies of single gene phylogenetic trees highlighted discrepancies which have implications for the classification of strains and new isolates; in particular, we propose the transfer of *Photorhabdus luminescens* subsp. *thracensis* to *Photorhabdus temperata* subsp. *thracensis* comb. nov. (type strain CIP 108426^T = DSM 15199^T). We found that, within the genus *Xenorhabdus*, strains or isolates that shared less than 97 % nucleotide identity (NI), calculated on the concatenated sequences of the four gene fragments (*recA*, *gyrB*, *dnaN* and *gltX*) encompassing 3395 nucleotides, did not belong to the same species. Thus, at the 97 % NI cutoff, we confirm the current 20 species of the genus *Xenorhabdus* and propose the description of a novel species, *Xenorhabdus vietnamensis* sp. nov. (type strain VN01^T = CIP 109945^T = DSM 22392^T). Within each of the three current species of the genus *Photorhabdus*, *P. asymbiotica*, *P. luminescens* and *P. temperata*, strains or isolates which shared less than 97 % NI did not belong to the same subspecies. Comparisons of the four gene fragments plus the *rplB* gene fragment analysed separately led us to propose four novel subspecies: *Photorhabdus luminescens* subsp. *caribbeanensis* subsp. nov. (type strain HG29^T = CIP 109949^T = DSM 22391^T), *P. luminescens* subsp. *hainanensis* subsp. nov. (type strain C8404^T = CIP 109946^T = DSM 22397^T), *P. temperata* subsp. *khanii* subsp. nov. (type strain C1^T = NC19^T = CIP 109947^T = DSM 3369^T), and *P. temperata* subsp. *tasmaniensis* subsp. nov. (type strain T327^T = CIP 109948^T = DSM 22387^T).

Abbreviations: NI, nucleotide identity; LGT, lateral gene transfer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Photorhabdus* strains FR33, IT12, FR32, KR04, BE09, Habana, T327^T, USCA01, E21, C8404^T, C8406, HG26, HG29^T and NZH3 are EU930333–EU930345 and FJ844932, respectively.

A table detailing primers used for DNA amplification and sequencing is available with the online version of this paper.

INTRODUCTION

The bacterial genera *Photorhabdus* and *Xenorhabdus* are phylogenetic sister groups belonging to the family *Enterobacteriaceae*; they are associated with entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively. The genus *Proteus* is their closest known phylogenetic neighbour (Yarza *et al.*, 2008; Suzuki *et al.*, 1996; <http://www.arb-silva.de/projects/living-tree/>). Phylogenetic analysis, based on 16S rRNA gene sequences, fails to resolve the phylogenetic relationships at the level of the deepest nodes of the trees within these two genera (Akhurst *et al.*, 2004; Rainey *et al.*, 1995; Tailliez *et al.*, 2006). This is probably because the phylogenetic information within these sequences, which evolve slowly due to stringent functional constraints, is insufficient. These phylogenetic trees should also reflect the rapid and simultaneous radiation of these micro-organisms that resulted from ecological events approximately 375 million years ago, which provided their ancestors with the opportunity to associate with entomopathogenic nematodes as a potential new environment to colonize (Poinar, 1993).

To address the genealogy of these entomopathogenic bacteria, we used an approach based on a multilocus sequence analysis as proposed by Gevers *et al.* (2005). Compared with single-gene phylogenies, the concatenation of several unlinked gene sequences should yield robust phylogenetic trees (Rokas *et al.*, 2003; Wertz *et al.*, 2003) by increasing the number of phylogenetically informative characters. A resolved phylogeny of these bacteria is required to study their co-evolution with their nematode hosts. Probable lateral gene transfers (LGT) can be identified by comparative analysis of the topology of single-gene phylogenetic trees (Doolittle, 1999) and such events have implications for the classification of strains and new isolates in the genera *Photorhabdus* and *Xenorhabdus*. We investigated the molecular phylogenies inferred from the sequences of each gene separately and from the concatenated sequences.

METHODS

Bacterial strains. The bacterial strains included in this study are listed in Table 1. *Xenorhabdus* strains were representatives of species with validly published or proposed names previously studied by Tailliez *et al.* (2006). *Photorhabdus* strains were part of our collection at INRA, Montpellier, France or were from the Collection de l'Institut Pasteur, Paris, France (CIP strains; <http://www.pasteur.fr/externe>). Before molecular analysis, all strains were grown, checked for purity and examined for the main characteristics of the genera *Photorhabdus* and *Xenorhabdus*, using the methods of Boemare & Akhurst (1988). Phenotypic characterization of the strains was as previously described (Tailliez *et al.*, 2006).

Total bacterial genomic DNA purification, DNA amplification and sequencing. Total bacterial genomic DNA was isolated from, and 16S rRNA gene sequences determined for, the following

Photorhabdus strains as described previously (Tailliez *et al.*, 2006): BE09, C8404^T, C8406, E21, FR32, FR33, Habana, HG26, HG29^T, IT12, KR04, NZH3, T327^T and USCA01. The 16S rRNA gene sequences of the other strains listed in Table 1 were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>). 16S rRNA gene, *recA*, *gyrB*, *dnaN*, *gltX* and *rplB* gene sequences from *Photorhabdus luminescens* subsp. *laumondii* strain TT01^T were obtained from the complete genome [GenBank accession number NC_005126]. Sequences of *Xenorhabdus nematophila* ATCC 19061^T and *Xenorhabdus bovienii* SS-2004 were obtained from the Donald Danforth Plant Science Center (<http://www.cns.fr/agc/microscope/home/index.php>) and those of *Photorhabdus asymbiotica* subsp. *asymbiotica* strain ATCC 43949 were obtained from the Sanger Institute (http://www.sanger.ac.uk/Projects/P_asymbiotica/). For the strains of our collection, DNA fragments of the *recA*, *gyrB*, *dnaN*, *gltX* and *rplB* genes were amplified using primers and annealing temperatures listed in Supplementary Table S1 (available in IJSEM Online). PCR was carried out in a GeneAmp 2400 thermal cycler PCR system (Perkin Elmer) programmed for 30 cycles of amplification: after an initial 5 min denaturation step at 94 °C, each cycle consisted of 1 min at 94 °C, 1 min at the annealing temperature corresponding to the primers selected and 2 min at 72 °C. For each locus, PCR primers were defined by aligning the two *Xenorhabdus* sequences (ATCC 19061^T and SS-2004) and the two *Photorhabdus* sequences (TT01^T and ATCC 43949). For the genus *Photorhabdus*, PCR fragments for all the strains studied were easily obtained using the first set of PCR primers defined. In contrast, for the genus *Xenorhabdus*, only a limited number of strains gave PCR fragments. These were sequenced and aligned. On the basis of these new alignments, more consensual PCR primers were then defined. Sequencing primers are presented in Supplementary Table S1. The sequences were obtained from Millegene, Toulouse, France (<http://www.millegen.com>). The five candidate genes studied and the reasons for their selection were as follows: (1) The *dnaN* gene (encoding a DNA polymerase III beta chain involved in DNA metabolism), the *gltX* gene (encoding a glutamyl-tRNA synthetase involved in protein synthesis) and the *rplB* gene (encoding the 50S ribosomal protein L2) are all single-copy orthologous genes resistant to horizontal transfer and their phylogeny in gammaproteobacteria reflects the relationships between bacterial species (Lerat *et al.*, 2003); (2) the *recA* gene (encoding a DNA recombination protein) and the *gyrB* gene (encoding a DNA gyrase beta subunit) have been used in several bacterial phylogeny studies (Akhurst *et al.*, 2004; Eisen, 1995; Hannula & Hänninen, 2007; Parkinson *et al.*, 2007; Thompson *et al.*, 2004; Wang *et al.*, 2007).

Sequence and phylogenetic analysis. Overlapping sequences for each gene were merged using the SeqMan software (<http://www.gatc-biotech.com/fr/software/lasergene/index.php>). The consensus sequences for each gene were aligned using the multiple sequence alignment program CLUSTAL W included in the BioEdit sequence alignment editor (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). After alignment, the 3' and 5' regions for which sequences were incomplete were truncated. The truncated sequences of *dnaN* (828 nucleotides), *gltX* (1057 nucleotides), *recA* (646 nucleotides) and *gyrB* (864 nucleotides) were concatenated and then aligned. Phylogenetic distance trees were calculated using the Kimura two-parameter model (Kimura, 1980) and the neighbour-joining module (Saitou & Nei, 1987) of PAUP software (Swofford, 2003). The *dnaN* and *gyrB* genes were mapped close together in the genomes of *X. bovienii* SS-2004, *X. nematophila* ATCC 19061^T, *P. asymbiotica* subsp. *asymbiotica* ATCC 43949 and *P. luminescens* subsp. *laumondii* TT01^T, whereas the *gltX*, *recA* and *rplB* genes are distributed differently depending on the genome. The sequences of the corresponding *Proteus vulgaris*,

Escherichia coli and *Yersinia pestis* genes were considered as outgroups. Sawyer's test for detecting recombination intervals based on the detection of shared patterns of polymorphisms (Sawyer, 1989) was performed with the computer program GENECONV (<http://www.math.wustl.edu/~sawyer>). The ratio between mean non-synonymous substitutions per non-synonymous site (dN) and mean synonymous substitutions per synonymous site (dS) was calculated by the method of Nei & Gojobori (1986) implemented in S.T.A.R.T. (Jolley *et al.*, 2001; <http://pubmlst.org/software/analysis/start2/>).

RESULTS AND DISCUSSION

Phylogeny of the genera *Photorhabdus* and *Xenorhabdus* based on the combination of four gene fragments: *recA*, *gyrB*, *dnaN* and *gltX*

The neighbour-joining distance tree, based on the concatenation of partial sequences of the four gene fragments *recA*, *gyrB*, *dnaN* and *gltX*, is presented in Fig. 1. 16S rRNA and *rplB* gene sequences were not concatenated together with these four gene sequences because they did not improve the robustness of the final phylogenetic tree. They were used for comparison. All the sequences of *Photorhabdus* and *Xenorhabdus* strains studied were distributed within two clades. One (C_P) included the sequences assigned to strains of the genus *Photorhabdus* and the other (C_X) included sequences of the genus *Xenorhabdus*. This distribution is in agreement with that from DNA–DNA hybridization data and phenotypic data for these two genera (Akhurst *et al.*, 1996; Boemare *et al.*, 1993; Fischer-Le Saux *et al.*, 1999), PCR-ribotyping (Brunel *et al.*, 1997; Fischer-Le Saux *et al.*, 1998) and 16S rRNA gene sequences (Fig. 2; Emelianoff *et al.*, 2008).

Within the *Photorhabdus* clade, three subclades (noted C_{P-I} , C_{P-II} and C_{P-III}) were clearly distinguished: these three lineages appear to have emerged simultaneously from one common ancestor. The phylogenetic node which linked the subclades *Photorhabdus luminescens* and *Photorhabdus temperata* (Fig. 1) had a low bootstrap value (0.63) and was only supported by the analysis of two genes, *recA* and *gltX* (Figs 3 and 4). The subclade C_{P-I} included the sequences of the species *P. luminescens*. Four *P. luminescens* subspecies of the five currently described were included in C_{P-I} : *P. luminescens* subsp. *akhurstii*; *P. luminescens* subsp. *kayaii*; *P. luminescens* subsp. *laumondii* and *P. luminescens* subsp. *luminescens*. For each of four genes – *recA* (Fig. 3), *gyrB* (Fig. 5), *dnaN* (Fig. 6) and *rplB* (Fig. 7) – the sequences of the *P. luminescens* subsp. *kayaii* type strain CIP 108428^T were similar to those of strains IT12, FR33, C8406 and KR04, indicating that these strains belong to the same subspecies. However, for the *gltX* gene, the sequence of the type strain was not closely related to those of the other four strains, suggesting that this sequence was probably obtained by LGT. Strain CIP 108426^T has been described as the type strain of *P. luminescens* subsp. *thracensis* on the basis of its 16S rRNA gene sequence (Hazir *et al.*, 2004). We also confirmed that the 16S rRNA

gene sequence of the type strain CIP 108426^T, kept in our collection at INRA Montpellier, was identical to that of the type strain for this subspecies deposited by Hazir *et al.* (2004) under the accession number AJ560634. This sequence and that of strain FR32 (recently isolated from infective juveniles captured using the *Galleria* trap in a cherry tree orchard near Avignon, France) were related to those of *P. luminescens* (Fig. 2). However, the analysis of their five gene sequences: *recA*, *gyrB*, *dnaN*, *gltX* and *rplB* (Figs 3–7) showed that these two strains were related to *P. temperata*. Thus, we propose the reclassification of strains CIP 108426^T and FR32 as *Photorhabdus temperata* subsp. *thracensis* comb. nov.

The subclade C_{P-II} included the sequences of the species *P. temperata* and *P. luminescens* subsp. *thracensis*. Our collection does not include *P. temperata* subsp. *cinerea* strains (Tóth & Lakatos, 2008) but the *gyrB* sequences available for these strains suggest that this subspecies should be included in subclade C_{P-II} (Fig. 5). Within C_{P-II} , four lineages were distinguished (plus a fifth for *P. temperata* subsp. *cinerea* for which we did not have the concatenated sequences). Two of them corresponded to two subspecies previously described: *P. temperata* subsp. *temperata* and *P. luminescens* subsp. *thracensis* (Fischer-Le Saux *et al.*, 1999; Hazir *et al.*, 2004). The two others included six strains (three strains each), five of them having previously been classified as *P. temperata* (Akhurst *et al.*, 1996, 2004). The four lineages were supported by the single gene phylogenetic analysis, including *rplB* (Figs 3–7). The three lineages corresponding to *P. temperata* strains were also confirmed by the 16S rRNA gene sequence analysis (Fig. 2). However, the 16S rRNA gene sequences of the strains *P. luminescens* subsp. *thracensis* CIP 108426^T and FR32 were related to those of the species *P. luminescens* (subclade C_{P-I} – see above).

Each of the four lineages described in the subclade C_{P-II} was from a different part of the world. Strains BE09, K122 and XINach^T were isolated from the north of Europe. Strains C1^T, Habana and Meg were isolated from the east coast of North America and Cuba. Strains T327^T, USCA01 and NZH3 were isolated around the Pacific Ocean. Strains CIP 106426^T and FR32 were isolated from the south of Europe and western part of Turkey. These four lineages can also be distinguished on the basis of their phenotypic traits (see the description of the subspecies below).

The subclade C_{P-III} included the sequences of the species *Photorhabdus asymbiotica* and that of strain Q614. The position of their *gyrB* sequences (Fig. 5; Kuwata *et al.*, 2008) suggests that strains Cbkj163 and Onlr40, isolated from Japanese heterorhabditid entomopathogenic nematodes, may also belong to this subclade. The *P. luminescens* subsp. *luminescens* strains Hb^T (Poinar *et al.*, 1977) and Hm (Bleakley & Nealson, 1988) showed 16S rRNA gene sequences related to those of *P. asymbiotica* subsp. *asymbiotica* (Fig. 2; Marokhazi *et al.*, 2003) whereas, for

Table 1. *Photorhabdus* and *Xenorhabdus* strains included in this study

Strain number	Previously classified as	Proposed classification	Nematode host species	Geographical origin	Source
9802892 ^T	<i>P. asymbiotica</i> subsp. <i>australis</i>	<i>P. asymbiotica</i> subsp. <i>australis</i>		Australia	R. Akhurst
3265-86 ^T	<i>P. asymbiotica</i> subsp. <i>asymbiotica</i>	<i>P. asymbiotica</i> subsp. <i>asymbiotica</i>		USA	R. Akhurst
FRG04 ^T	<i>P. luminescens</i> subsp. <i>akhurstii</i>	<i>P. luminescens</i> subsp. <i>akhurstii</i>	<i>H. indica</i>	Guadeloupe	Fischer-Le Saux
CIP 108428 ^T	<i>P. luminescens</i> subsp. <i>kayaii</i>	<i>P. luminescens</i> subsp. <i>kayaii</i>	<i>Heterorhabditis</i> sp.	Turkey	CIP
C8406	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>kayaii</i>	<i>Heterorhabditis</i> sp.	China	R. Akhurst
FR33	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>kayaii</i>	Not yet defined	France	Domaine expérimental La Tapy
IT12	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>kayaii</i>	<i>H. bacteriophora</i>	Italy	E. Tarasco
KR04	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>kayaii</i>	<i>Heterorhabditis</i> sp.	Korea	H. Choo
E21	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>laumondii</i>	<i>H. bacteriophora</i>	Australia	G. Poinar
Hb ^T	<i>P. luminescens</i> subsp. <i>luminescens</i>	<i>P. luminescens</i> subsp. <i>luminescens</i>	<i>H. bacteriophora</i>	Australia	R. Akhurst
Hm	<i>P. luminescens</i> subsp. <i>luminescens</i>	<i>P. luminescens</i> subsp. <i>luminescens</i>	<i>H. bacteriophora</i>	USA	R. Akhurst
CIP 108426 ^T	<i>P. luminescens</i> subsp. <i>thracensis</i>	<i>P. temperata</i> subsp. <i>thracensis</i>	<i>Heterorhabditis</i> sp.	Turkey	CIP
FR32	<i>P. luminescens</i>	<i>P. temperata</i> subsp. <i>thracensis</i>	Not yet defined	France	Domaine expérimental La Tapy
XINach ^T	<i>P. temperata</i> subsp. <i>temperata</i>	<i>P. temperata</i> subsp. <i>temperata</i>	<i>H. megidis</i>	Russia	R. Akhurst
BE09	<i>P. temperata</i> subsp. <i>temperata</i>	<i>P. temperata</i> subsp. <i>temperata</i>	<i>H. megidis</i>	Belgium	M. Moens
K122	<i>P. temperata</i> subsp. <i>temperata</i>	<i>P. temperata</i> subsp. <i>temperata</i>	<i>H. megidis</i>	Ireland	D. Clark
Cl ^T (=NC19 ^T)	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>khanii</i>	<i>H. heliothidis</i>	USA	N. Boemare
Habana	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>khanii</i>	<i>Heterorhabditis</i> sp.	Cuba	R. Akhurst
Meg	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>khanii</i>	<i>H. megidis</i>	USA	R. Akhurst
NZH3	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>tasmaniensis</i>	<i>H. zealandica</i>	New Zealand	R. Akhurst
T327 ^T	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>tasmaniensis</i>	<i>H. zealandica</i>	Australia	R. Akhurst
USCA01	<i>P. temperata</i>	<i>P. temperata</i> subsp. <i>tasmaniensis</i>	<i>H. marelatus</i>	USA	P. Stock
Q614	<i>Photorhabdus</i> sp.	<i>Photorhabdus</i> sp.	<i>Heterorhabditis</i> sp.	Australia	R. Akhurst
C8404 ^T	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>hainanensis</i>	<i>Heterorhabditis</i> sp.	China	R. Akhurst
HG26	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>caribbeanensis</i>	<i>Heterorhabditis</i> sp.	St Martin	H. Mauléon
HG29 ^T	<i>P. luminescens</i>	<i>P. luminescens</i> subsp. <i>caribbeanensis</i>	<i>H. bacteriophora</i>	Guadeloupe	H. Mauléon
Q58 ^T	<i>X. beddingii</i>	<i>X. beddingii</i>	Unknown	Australia	R. Akhurst
T228 ^T	<i>X. bovienii</i>	<i>X. bovienii</i>	<i>S. feltiae</i>	Australia	R. Akhurst
CS03	<i>X. bovienii</i>	<i>X. bovienii</i>	<i>S. weiseri</i>	Czech Republic	Z. Mráček
Si	<i>X. bovienii</i>	<i>X. bovienii</i>	<i>S. intermedium</i>	USA	R. Akhurst
TB20	<i>X. bovienii</i>	<i>X. bovienii</i>	<i>S. sichuanense</i>	China	Z. Mráček
USNY95	<i>X. bovienii</i>	<i>X. bovienii</i>	<i>S. kraussei</i>	USA	P. Stock
DSM 16342 ^T	<i>X. budapestensis</i>	<i>X. budapestensis</i>	<i>S. bicornutum</i>	Serbia	DSMZ
CN03	<i>X. budapestensis</i>	<i>X. budapestensis</i>	<i>S. ceratophorum</i>	China	P. Stock
DSM 16337 ^T	<i>X. ehlersii</i>	<i>X. ehlersii</i>	<i>S. serratum</i> *	China	DSMZ
DSM 17382 ^T	<i>X. indica</i>	<i>X. indica</i>	<i>S. thermophilum</i>	India	DSMZ
OM01	<i>X. indica</i>	<i>X. indica</i>	<i>S. abbasi</i>	Sultanate of Oman	S. Elawad
DSM 16336 ^T	<i>X. innexi</i>	<i>X. innexi</i>	<i>S. scapterisci</i>	Uruguay	DSMZ
UY61	<i>X. innexi</i>	<i>X. innexi</i>	<i>S. scapterisci</i>	Uruguay	G. Smart
DSM 16522 ^T	<i>X. japonica</i>	<i>X. japonica</i>	<i>S. kushidai</i>	Japan	DSMZ
G6 ^T	<i>X. poinarii</i>	<i>X. poinarii</i>	<i>S. glaseri</i>	USA	R. Akhurst
AZ26	<i>X. poinarii</i>	<i>X. poinarii</i>	<i>S. glaseri</i>	Portugal	N. Simões
CU01	<i>X. poinarii</i>	<i>X. poinarii</i>	<i>S. cubanum</i>	Cuba	E. Arteaga
DSM 16338 ^T	<i>X. szentirmaii</i>	<i>X. szentirmaii</i>	<i>S. rarum</i>	Argentina	DSMZ
KE01 ^T	<i>X. hominickii</i>	<i>X. hominickii</i>	<i>S. kari</i>	Kenya	P. Stock
KR01	<i>X. hominickii</i>	<i>X. hominickii</i>	<i>S. monticolum</i>	Korea	P. Stock
KR05	<i>X. hominickii</i>	<i>X. hominickii</i>	<i>S. monticolum</i>	Korea	P. Stock
SaV ^T	<i>X. kozodoii</i>	<i>X. kozodoii</i>	<i>S. arenarium</i>	Russia	R. Akhurst
ES01	<i>X. kozodoii</i>	<i>X. kozodoii</i>	Not yet defined	Spain	O. Triggiani
IT10	<i>X. kozodoii</i>	<i>Xenorhabdus</i> species	<i>S. apuliae</i>	Italy	O. Triggiani
FRM16 ^T	<i>X. doucetiae</i>	<i>X. doucetiae</i>	<i>S. diaprepesi</i>	Martinique	H. Mauléon
USTX62 ^T	<i>X. cabanillasii</i>	<i>X. cabanillasii</i>	<i>S. riobrave</i>	USA	E. Cabanillas
ID10 ^T	<i>X. griffinae</i>	<i>X. griffinae</i>	<i>S. hermaphroditum</i>	Indonesia	C. Griffin

Table 1. cont.

Strain number	Previously classified as	Proposed classification	Nematode host species	Geographical origin	Source
PR06-A ^T	<i>X. romanii</i>	<i>X. romanii</i>	<i>S. puertoricense</i>	Puerto Rico	H. Mauléon
Q1 ^T	<i>X. miraniensis</i>	<i>X. miraniensis</i>	Steinernematidae	Australia	R. Akhurst
TH01 ^T	<i>X. stockiae</i>	<i>X. stockiae</i>	<i>S. siamkayai</i>	Thailand	P. Stock
USNJ01 ^T	<i>X. koppenhoeferi</i>	<i>X. koppenhoeferi</i>	<i>S. scarabaei</i>	USA	A. Koppenhöfer
VC01 ^T	<i>X. mauleonii</i>	<i>X. mauleonii</i>	Unknown	St. Vincent	H. Mauléon
VN01 ^T	<i>Xenorhabdus</i> sp.	<i>X. vietnamensis</i>	<i>S. sangi</i>	Vietnam	P. Stock

* *S. serratum*=*S. longicaudum*.

the five other genes studied, the sequences of these two strains were clearly grouped in the subclade C_{P-I} (Figs 3–7).

Within the *Xenorhabdus* clade, we propose four subclades noted C_{X-I} to C_{X-IV}. The subclade C_{X-I} included eight species of the genus *Xenorhabdus* which are able to grow at temperatures of 35–42 °C, except *Xenorhabdus japonica* DSM 16522^T (maximum temperature for growth, 34 °C) (Tailliez *et al.*, 2006). Analysis of *recA*, *dnaN* and *gltX* gene sequences supported this subclade; however, the *gyrB* gene sequences did not, the *gyrB* sequence of *Xenorhabdus romanii* strain PR06-A^T being similar to that of *Xenorhabdus beddingii* Q58^T. For *gyrB*, the lineage leading to the species *Xenorhabdus poinarii* was rejected at a basal position within the *Xenorhabdus* clade. The phylogeny of the subclade C_{X-II} was not fully resolved except between: (1) *X. nematophila* ATCC 19061^T and *Xenorhabdus koppenhoeferi* USNJ01^T [two species which have a maximum growth temperature below 35 °C (Tailliez *et al.*, 2006)] and (2) *X. beddingii* strain Q58^T and *Xenorhabdus miraniensis* strain Q1^T both isolated in Australia. Subclade C_{X-III} was composed of only one lineage leading to the species *X. bovienii*. Subclade C_{X-IV} covered five species able to grow at temperatures of 35–42 °C. There was strong support for most of the phylogenetic nodes of this subclade, suggesting a common ancestor at the basal position of the *Xenorhabdus* clade. The capacity to grow at temperatures >35 °C may be an ancestral character lost by a few species, for example *X. bovienii* (C_{X-III}), *Xenorhabdus hominickii*, *X. koppenhoeferi* and *X. nematophila* (C_{X-II}), and *X. japonica* (C_{X-I}).

We calculated nucleotide identity (NI) between strains for the concatenated sequences of the four gene fragments (*recA*, *gyrB*, *dnaN* and *gltX*) encompassing 3395 nucleotides. We found that, within the genus *Xenorhabdus*, strains or isolates which shared less than 97 % NI do not belong to the same species. For example, within *X. bovienii* and *X. poinarii*, the NI between the strains studied was more than 98 % (NI=100 % between the three strains of *X. hominickii*, one of them isolated in Kenya and the other two in Korea). Between *Xenorhabdus budapestensis* and *Xenorhabdus cabanillasii*, two closely related species, the NI is 96.9 %. Thus, at the 97 % NI cutoff, we confirm the existing classification of 20 species of the genus *Xenorhabdus* proposed on the basis of published molecular and phenotypic data

(Akhurst & Boemare, 1988; Lengyel *et al.*, 2005; Somvanshi *et al.*, 2006; Tailliez *et al.*, 2006). We propose that strain VN01^T should be considered as a representative of a new species, *Xenorhabdus vietnamensis* sp. nov., with the closest species being *X. japonica*. We consider strain IT10 to be at the limit of the species *Xenorhabdus kozodoii* (NI=96.7 %): it should, therefore, not be assigned to this species.

Similarly, within the three currently recognized species *P. luminescens*, *P. temperata* and *P. asymbiotica*, strains or isolates which shared less than 97 % NI calculated on the concatenated sequences do not belong to the same subspecies. There was one exception: *P. luminescens* subsp. *kayaii* strain CIP 108428^T, for which a LGT involving *gltX* was detected leading to an erroneous phylogenetic position of this strain in the tree based on concatenated sequences. The species *P. luminescens* and *P. temperata* are heterogeneous. The DNA relatedness between two *P. luminescens* subspecies ranges from 48 to 72 % and the DNA–DNA relatedness between *P. temperata* subsp. *temperata* and *P. temperata* is around 51–54 % (Fischer-Le Saux *et al.*, 1999). These values are less than 70 %, which was the threshold used in the 1980s–1990s for the definition of a bacterial species (Wayne *et al.*, 1987). For *P. asymbiotica*, the DNA relatedness data available between the two subspecies are restricted to the comparison of only two isolates (Akhurst *et al.*, 2004). We consider here that the *P. luminescens* and *P. temperata* subspecies are ‘equivalent’ to species and that the 97 % NI cutoff can be used in the same way as for the genus *Xenorhabdus*.

On the basis of the groupings obtained with the four gene fragments plus the *rplB* gene fragment, we propose (1) the transfer of *P. luminescens* subsp. *thracensis* to *P. temperata* subsp. *thracensis* comb. nov. and (2) four new subspecies: *P. luminescens* subsp. *caribbeanensis* subsp. nov., *P. luminescens* subsp. *hainanensis* subsp. nov., *P. temperata* subsp. *khanii* subsp. nov., and *P. temperata* subsp. *tasmaniensis* subsp. nov.

Gene evolution within the genera *Photorhabdus* and *Xenorhabdus*

Potential gene transfer events and subsequent recombination can be identified using multigene-based phylogeny. The presence of genes whose phylogenetic relationships are not congruent with the relationship inferred from other

Fig. 1. Phylogenetic tree derived from distance analysis of four concatenated protein-coding sequences (*recA*, *gyrB*, *dnaN*, *gltX*). Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The concatenated sequences of *Escherichia coli* and *Yersinia pestis* are used as outgroups. Bootstrap values (percentages of 1000 replicates) (Felsenstein, 1988) of more than 50 % are shown at the nodes. Clades (C_P, C_X) and subclades are shown. Bar, 10 % sequence divergence. *The phylogenetic position of strain CIP 108428^T outside of the group *P. luminescens* subsp. *kayaii* is linked to a likely lateral gene transfer of the *gltX* sequence.

genes suggests that these genes have different evolutionary histories and that LGT has occurred between lineages (Doolittle, 1999).

Our single-gene phylogenetic tree topologies provided no evidence of LGT between the genera *Photorhabdus* and *Xenorhabdus* (Figs 2–7) for any of the six genes studied. For our phylogenetic study, we selected genes resistant to LGT (Lerat *et al.*, 2003), and Sawyer's test for recombination applied to our sequence data did not highlight putative recombination events for *recA*, *dnaN*, *gltX*, *gyrB* and *rplB*, validating our selection except for the *gltX* gene sequence of strain CIP 108426^T (see below). If incongruence in the gene trees is indeed a product of an ancient or difficult to detect LGT, then it appears to have gone to fixation. In contrast, our results suggested lateral transfer of the 16S rRNA gene within the genera *Photorhabdus* and *Xenorhabdus*. Phylogenetic analysis based on the 16S rRNA gene (Marokhazi *et al.*, 2003; Fig. 2) suggests a common ancestor for *P. luminescens* subsp. *luminescens* strains Hb^T and Hm and *P. asymbiotica* subsp. *asymbiotica* strains 3265-86^T and ATCC 43949, but this relationship did not appear from the phylogenetic analysis of the *recA*, *gyrB*, *dnaN* and *gltX* genes; according to the sequences of these four genes, strains Hb^T and Hm are in the *P. luminescens* subclade C_{P-I}. Similarly, strains CIP 108426^T and FR32 were related to *P. luminescens* according to 16S rRNA gene analysis (Fig. 2) and strain CIP 108426^T was proposed as the type strain of the subspecies *P. luminescens* subsp. *thracensis* (Hazir *et al.*, 2004). However, our multigene analysis classified these strains as *P. temperata* (Figs 3–7). Sawyer's test indicates that strains CIP 108426^T and FR32 share a 286 bp 16S fragment (position 764–1049 including 28 polymorphic sites) with *P. luminescens* subsp. *kayaii* strains KR04 and C8406 and a 527 bp 16S fragment (position 389–915 including 28 polymorphic sites) with *P. temperata* subsp. *temperata* strain K122, resulting in 16S mosaic genes and explaining the ambiguous phylogenetic position of these two strains. The phylogenetic relationship of the *gltX* gene sequence from *P. luminescens* subsp. *kayaii* strain CIP 108428^T was not the same as that from the other gene sequences (16S rRNA, *recA*, *gyrB*, *dnaN* and *rplB*), suggesting a more recent LGT restricted to this strain only. This sequence was checked by independent repeated sequencing and confirmed. Sawyer's test shows that the *gltX* gene sequence of strain CIP 10428^T is a mosaic sequence sharing fragments with the gene sequences of strains KR04 and C8406 (position 1–212 including 52 polymorphic sites), IT12 (position 17–371 including 91 polymorphic sites) and FR33 (position 1–383 including

101 polymorphic sites) but also with that of *P. temperata* subsp. *thracensis* comb. nov. strains CIP 108426^T and FR32 (position 499–1073 including 159 polymorphic sites). This resulted in an erroneous phylogenetic position for this strain outside the group composed of *P. luminescens* subsp. *kayaii* strains IT12, FR33, C8406 and KR04 (Figs 1 and 4). For the genus *Xenorhabdus*, the phylogenetic analysis suggests a common ancestor for *X. romanii* PR06-A^T and *Xenorhabdus doucetiae* FRM16^T for all the genes tested except for *gyrB*, for which *X. romanii* shares a common ancestor with *X. beddingii* Q58^T (Fig. 5). The basal position of *X. poinarii* inferred by the *gyrB* sequences (Fig. 5) was not inferred by the other gene sequences (*recA*, *dnaN* and *gltX*; Figs 3, 6 and 4). Sawyer's test also suggests putative recombination events in the 3' end (position 1070–1378 including 36 polymorphic sites) of the 16S rRNA gene sequence between *X. bovienii*, *X. hominickii*, *X. nematophila*, *X. innexi* and *X. stockiae*.

Although lateral transfer of rRNA genes was previously thought unlikely (Jain *et al.*, 1999), numerous examples of lateral transfers for rRNA gene segments, and entire rRNA genes and operons have been reported in several bacterial groups. Evidence for lateral transfer of 16S rRNA gene segments has been reported for Rhizobiaceae, Actinomycetes, between species of the *Streptococcus anginosus* group and in species of the genus *Aeromonas* (Eardly *et al.*, 1996; Schouls *et al.*, 2003; Sneath, 1993; van Berkum *et al.*, 2003; Wang & Zhang, 2000). Whole 16S rRNA gene exchanges were suggested in species of the genus *Helicobacter* (Dewhirst *et al.*, 2005) and horizontal transfer of an entire rRNA operon was also proposed by Yap *et al.* (1999) in the Actinomycete *Thermomonospora chromogena*. Various factors may favour LGT and homologous recombination of rRNA genes in the genera we are studying: the potential co-infection of individual insects with several entomopathogenic nematodes; massive population growth of their symbiotic bacteria associated with septicaemia; and the substantial sequence similarities between the 16S rRNA genes in these closely related bacteria. Sergeant *et al.* (2006) used a similar multilocus sequence analysis including four housekeeping genes and suggested that the *serC* locus – encoding a phosphoserine aminotransferase – was also subjected to frequent recombination in *Xenorhabdus* genomes.

Discrepancies in individual gene trees could also be the result of different evolutionary pressures on the genes studied. For the genera *Photorhabdus* and *Xenorhabdus*, the dN/dS ratios are lower than 1 for all the genes analysed (Table 2), indicating that they are under purifying pressure

Fig. 2. Phylogenetic tree derived from distance analysis of 16S rRNA gene sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The 16S rRNA gene sequences of *Proteus vulgaris* [AJ301683], *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. Bar, 1 % sequence divergence. 1121^T=CIP 108428^T; 39-8^T=CIP 108426^T; ITH-LA3=IT12; NC19^T=C1^T; ATCC 43950^T=3265-86^T.

Fig. 3. Phylogenetic tree derived from distance analysis of *recA* sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The *recA* sequences of *Proteus vulgaris* [X55555], *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. GenBank accession numbers of the sequences are in brackets. Bar, 10 % sequence divergence.

Fig. 4. Phylogenetic tree derived from distance analysis of *gltX* sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The *gltX* sequences of *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. GenBank accession numbers of the sequences are in brackets. Bar, 10 % sequence divergence. *The erroneous phylogenetic position of strain CIP 108428^T outside of the group *P. luminescens* subsp. *kayaii* is linked to a likely lateral gene transfer of the *gltX* sequence.

such that most amino acid substitutions are deleterious. Mean non-synonymous substitutions per non-synonymous site (dN) and mean synonymous substitutions per

synonymous site (dS) are higher for *Xenorhabdus* than for *Photobacterium* suggesting that the genus *Xenorhabdus* evolves faster on an absolute timescale than the genus

Fig. 5. Phylogenetic tree derived from distance analysis of *gyrB* sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The *gyrB* sequences of *Proteus vulgaris* [CAC69413], *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. GenBank accession numbers of the sequences are in brackets. Bar, 10 % sequence divergence.

Photorhabdus at both non-synonymous and synonymous sites and for the five genes analysed. In addition, we also found variation in dN and dS between genes and within the genera between subclades. This heterogeneity should also contribute in part to the different tree topologies observed.

At least, the sequences studied are highly conserved (similarity >80 % within the genus *Xenorhabdus* and >85 % within the genus *Photorhabdus* for *recA*, *gyrB*, *dnaN* and *gltX*; similarity >92 % for *rplB* and >95 % for 16S rRNA gene sequences within both genera). Thus, a low phylogenetic signal to noise

Fig. 6. Phylogenetic tree derived from distance analysis of *dnaN* sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The *dnaN* sequences of *Proteus vulgaris* [AP004237], *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. GenBank accession numbers of the sequences are in brackets. Bar, 10 % sequence divergence.

ratio should also contribute to the lack of resolution of the single gene trees. This observation is particularly true for the 16S rRNA and *rplB* gene sequences for which the deepest nodes of the phylogenetic trees are not resolved (Figs 2 and 7).

The sequences of these two genes were not included in the concatenated tree because they do not improve the resolution at the deepest nodes of the tree. This hypothesis of low phylogenetic signal to noise ratio is supported by the increased

Fig. 7. Phylogenetic tree derived from distance analysis of *rplB* sequences. Kimura two-parameter distances were used to reconstruct the neighbour-joining tree. The *rplB* sequences of *Escherichia coli* [obtained from NC_000913] and *Yersinia pestis* [obtained from NC_003143] are used as outgroups. Bootstrap values (percentages of 1000 replicates) of more than 50 % are shown at the nodes. GenBank accession numbers of the sequences are in brackets. Bar, 10 % sequence divergence.

resolution of the phylogenetic tree based on the four concatenated sequences (Rokas *et al.*, 2003; Wertz *et al.*, 2003).

Thus, great care must be taken for taxonomic and phylogenetic studies of these two genera; we recommend the use of a multigene approach for avoiding misclassification of new isolates.

Description of *Xenorhabdus vietnamensis* sp. nov.

Xenorhabdus vietnamensis [vi.et.nam.en'sis N.L. fem. adj. *vietnamensis* from Vietnam, the source of the nematode *Steinernema sangi* (Phan *et al.*, 2001) from which the type strain was isolated].

Table 2. Mean non-synonymous (dN) and mean synonymous (dS) divergences between sequences representing the genera *Photorhabdus*, *Xenorhabdus* and their subclades

SD, Standard deviation.

	Mean dN (SD)	Mean dS (SD)	dN/dS
<i>rplB</i>			
<i>Photorhabdus</i>	0.003 (0.002)	0.185 (0.089)	0.016
C _{P-I}	0.001 (0.001)	0.051 (0.045)	0.026
C _{P-II}	0	0.036 (0.022)	0
C _{P-III}	0.003 (0.002)	0.113 (0.098)	0.023
<i>Xenorhabdus</i>	0.007 (0.003)	0.206 (0.050)	0.034
C _{X-I}	0.007 (0.004)	0.167 (0.052)	0.040
C _{X-II}	0.005 (0.003)	0.215 (0.043)	0.024
C _{X-III}	0.001 (0.001)	0.026 (0.009)	0.031
C _{X-IV}	0.005 (0.003)	0.150 (0.077)	0.034
<i>recA</i>			
<i>Photorhabdus</i>	0.003 (0.002)	0.366 (0.199)	0.008
C _{P-I}	0	0.145 (0.078)	0
C _{P-II}	0.001 (0.001)	0.085 (0.051)	0.014
C _{P-III}	0	0.106 (0.092)	0
<i>Xenorhabdus</i>	0.006 (0.004)	0.765 (0.259)	0.008
C _{X-I}	0.002 (0.001)	0.389 (0.127)	0.005
C _{X-II}	0.002 (0.002)	0.549 (0.257)	0.004
C _{X-III}	0.001 (0.001)	0.085 (0.034)	0.010
C _{X-IV}	0	0.259 (0.227)	0
<i>gyrB</i>			
<i>Photorhabdus</i>	0.013 (0.013)	0.485 (0.230)	0.028
C _{P-I}	0.038 (0.055)	0.312 (0.211)	0.121
C _{P-II}	0.004 (0.003)	0.173 (0.104)	0.023
C _{P-III}	0.004 (0.002)	0.234 (0.122)	0.017
<i>Xenorhabdus</i>	0.021 (0.008)	0.615 (0.192)	0.034
C _{X-I}	0.020 (0.008)	0.558 (0.212)	0.036
C _{X-II}	0.012 (0.007)	0.418 (0.202)	0.029
C _{X-III}	0.003 (0.002)	0.053 (0.029)	0.051
C _{X-IV}	0.012 (0.007)	0.431 (0.254)	0.028
<i>dnaN</i>			
<i>Photorhabdus</i>	0.009 (0.005)	0.381 (0.175)	0.023
C _{P-I}	0.003 (0.002)	0.170 (0.093)	0.019
C _{P-II}	0.003 (0.002)	0.091 (0.057)	0.033
C _{P-III}	0.001 (0.001)	0.159 (0.023)	0.007
<i>Xenorhabdus</i>	0.017 (0.005)	1.022 (0.570)	0.016
C _{X-I}	0.014 (0.005)	0.527 (0.176)	0.028
C _{X-II}	0.007 (0.003)	0.552 (0.260)	0.013
C _{X-III}	0.002 (0.001)	0.065 (0.020)	0.036
C _{X-IV}	0.011 (0.006)	0.447 (0.284)	0.024
<i>gltX</i>			
<i>Photorhabdus</i>	0.015 (0.007)	0.370 (0.209)	0.041
C _{P-I}	0.011 (0.005)	0.233 (0.120)	0.046
C _{P-II}	0.006 (0.005)	0.093 (0.060)	0.061
C _{P-III}	0.015 (0.005)	0.356 (0.081)	0.042
<i>Xenorhabdus</i>	0.191 (0.375)	0.919 (0.379)	0.208
C _{X-I}	0.346 (0.477)	0.824 (0.555)	0.420
C _{X-II}	0.243 (0.426)	0.901 (0.394)	0.270
C _{X-III}	0.003 (0.002)	0.068 (0.029)	0.045
C _{X-IV}	0.019 (0.008)	0.468 (0.271)	0.041

Growth in LB broth is stopped by temperatures above 37 °C. In contrast to the closely related species *X. japonica*, colonies are pink, hydrolyse aesculin, show a lecithinase activity, produce acid from trehalose and assimilate putrescine, but not D-alanine (Tailliez *et al.*, 2006).

The type strain is VN01^T (=CIP 109945^T =DSM 22392^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ205447.

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

Photorhabdus temperata subsp. *khanii* [kha'ni.i N.L. gen. masc. n. *khanii* in honour of A. Khan, who described the nematode *Chromonema heliothidis* (Khan *et al.*, 1976) =*Heterorhabditis bacteriophora* (Poinar, 1975) recovered from prepupal and pupal specimens of the corn earworm, *Heliothis zea*, from Clayton, North Carolina, 1971].

Growth in LB broth is stopped by temperatures above 35–36 °C. Colonies are pigmented and produce light. Acid is produced from salicin and aesculin but not from L-fucose. Myo-inositol is assimilated but *cis*-aconitate is not.

The type strain C1^T (=NC19^T =CIP 109947^T =DSM 3369^T) is symbiotically associated with *Heterorhabditis bacteriophora* (North Carolina; Marokhazi *et al.*, 2003). Strains Meg and Habana are also representatives of this subspecies. The GenBank accession number of the 16S rRNA gene sequence of the type strain is AY278657.

Description of *P. temperata* subsp. *tasmaniensis* subsp. nov.

P. temperata subsp. *tasmaniensis* [tas.ma.ni.en'sis N.L. fem. adj. *tasmaniensis* from Tasmania (Australia), the source of the nematode *Heterorhabditis zealandica* from which the type strain was isolated].

Growth in LB broth is stopped by temperatures above 33–34 °C. Colonies are yellow, except those of strain T327^T, and produce light. Produces acid from trehalose but not from salicin. *Cis*-aconitate, citrate and D-glucuronate are assimilated.

The type strain is T327^T (=CIP 109948^T =DSM 22387^T). Strains USCA01 and NZH3 are also representatives of this subspecies. The GenBank accession number of the 16S rRNA gene sequence of the type strain is EU930339.

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

Photorhabdus luminescens subsp. *hainanensis* [ha.i.nan.en'sis. N.L. fem. adj. *hainanensis* from the Hainan island (China), the source of the nematode (*Heterorhabditis* species) from which the type strain was isolated].

Growth in LB broth is stopped by temperatures above 37–38 °C. Colonies are orange and produce light, indole and acid from 5-keto-gluconate but not from gluconate. D-

Mannitol, L-histidine and α -(-)-L-fucose are assimilated but propionate and (+)-trehalose are not.

The type strain is C8404^T (=CIP 109946^T =DSM 22397^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is EU930342.

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

Photorhabdus luminescens subsp. *caribbeanensis* [ca.rib.be.a-n.en'sis. N.L. fem. adj. from the Caribbean islands (Guadeloupe), the source of the nematode *Heterorhabditis bacteriophora* from which the type strain was isolated].

Growth in LB broth is stopped by temperatures above 38–39 °C. Colonies produce light. Strain HG29^T is not pigmented, whereas strain HG26 is pink. Positive for gelatinase activity and negative for lecithinase activity. Assimilates and produces acid from many carbohydrates, including ribose, fructose, mannitol, N-acetylglucosamine and maltose. Assimilates Simmons' citrate.

The type strain is HG29^T (=CIP 109949^T =DSM 22391^T). Strain HG26 is also representative of this subspecies. The GenBank accession number of the 16S rRNA gene sequence of the type strain is EU930345.

Description of *Photorhabdus temperata* subsp. *thracensis* (Hazir *et al.* 2004) Tailliez, Laroui, Ginibre, Paule, Pagès and Boemare comb. nov.

Photorhabdus temperata subsp. *thracensis* (thra.cen'sis. L. fem. adj. *thracensis* referring to Thracia, Thrace, Western part of Turkey from where the type strain has been isolated).

Basonym: *Photorhabdus luminescens* subsp. *thracensis* Hazir *et al.* 2004.

Growth in LB broth is stopped by temperatures above 36–37 °C. Colonies are not pigmented. The type strain obtained from the Collection de l'Institut Pasteur is a phase II variant which does not produce light, in contrast to isolate FR32. Positive for the Voges–Proskauer and Christensen's urease tests. Assimilates *cis*-aconitate and does not produce acid from ribose.

The type strain is CIP 108426^T (=DSM 15199^T). Strain FR32 is also representative of this subspecies.

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