JOURNAL OF BACTERIOLOGY, July 2004, p. 4376–4381 Vol. 186, No. 13
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.13.4376–4381.2004

Variation in the Effectors of the Type III Secretion System among *Photorhabdus* Species as Revealed by Genomic Analysis

Karine Brugirard-Ricaud,[1] Alain Givaudan,[1] Julian Parkhill,[2] Noel Boemare,[1] Frank Kunst,[3] Robert Zumbihl,[1]* and Eric Duchaud[1]

Laboratoire EMIP Ecologie Microbienne des Insectes et Interaction Hôte-Pathogène, Université de Montpellier II, UMR1133 INRA-UMII, 34095 Montpellier Cedex 5,[1] and Laboratoire de Génomique des Microorganismes Pathogènes, Institut Pasteur, 75724 Paris Cedex 15,[3] France, and The Sanger Institute, Hinxton, Cambridge CB10 1SA, United Kingdom[2]

Received 20 January 2004/Accepted 17 March 2004

Entomopathogenic bacteria of the genus *Photorhabdus* harbor a type III secretion system. This system was probably acquired prior to the separation of the species within this genus. Furthermore, the core components of the secretion machinery are highly conserved but the predicted effectors differ between *Photorhabdus luminescens* and *P. asymbiotica*, two highly related species with different hosts.

Bacterial pathogens have evolved complex mechanisms to invade hosts, to escape host defenses, to multiply, and, finally, to cause harm to their hosts (6, 20). According to Wassenaar and Gaastra (27), virulence genes are those directly responsible for pathological damage caused by pathogens and are normally absent from their nonpathogenic relatives. These genes can be organized in so-called pathogenicity islands that are large genomic regions, often unstable and probably acquired during evolution via horizontal genetic transfer (14, 17). This feature became particularly apparent for a set of approximately 20 genes that together encode a pathogenicity device called the type III secretion system (TTSS) (29) whose central function is the delivery of bacterial proteins into eukaryotic cells (3). More than 20 TTSSs have been discovered so far in gram-negative bacteria pathogenic for mammals and plants (3, 15) but also in bacterial symbionts of plants and insects (4, 5, 25).

Photorhabdus and *Xenorhabdus* (members of the *Enterobacteriaceae* family) (2, 12) are bacterial symbionts of entomopathogenic nematodes belonging to the families *Heterorhabditidae* and *Steinernematidae*, respectively. These bacteria are transported by their nematode vectors into the hemocoel of the insect host, which is quickly killed by a combination of toxin action and septicemia (12). Three species were defined in the genus *Photorhabdus*: *Photorhabdus luminescens*, *P. temperata*, and *P. asymbiotica* (11). In addition, *P. luminescens* and *P. temperata* are subdivided into subspecies as follows: *P. luminescens* subsp. *luminescens*, *P. luminescens* subsp. *akhurstii*, and *P. luminescens* subsp. *laumondii* and *P. temperata* subsp. *temperata* (11). *P. asymbiotica* was never found associated with entomopathogenic nematodes but is isolated from human infections (8, 19). Recently, Akhurst et al. have proposed two subspecies for *P. asymbiotica*: *P. asymbiotica* for an American clinical strain and *P. australis* for an Australian clinical strain (1).

* Corresponding author. Mailing address: Laboratoire EMIP Ecologie Microbienne des Insectes et Interaction Hôte-Pathogène, Université de Montpellier II, UMR1133 INRA-UMII, 34095 Montpellier Cedex 5, France. Phone: 0033 4 671446 66. Fax: 0033 4 671446 79. E-mail: zumbihl@thor.univ-montp2.fr.

The recent identification of TTSSs in *P. luminescens* (7, 28, 31) prompted us to analyze the genomic diversity of TTSS within the genus *Photorhabdus*.

Comparative genomics of TTSS organization in *Photorhabdus*. Comparisons of the genomic organization of the *Photorhabdus* TTSSs (Fig. 1) were performed with three strains: *P. luminescens* subsp. *laumondii* (strain TT01) (http://genolist.pasteur.fr/PhotoList/) (7), *P. luminescens* subsp. *akhurstii* (strain W14; accession number AY144116) (28), and *P. asymbiotica* (strain ATCC43949), for which the genome sequence is being determined at the Sanger Center (http://www.sanger.ac.uk/Projects/P_asymbiotica/). We identified a TTSS-encoding locus in all three strains analyzed. Our analyses revealed, first, identical TTSS backbones, including all the genes predicted to encode the injectisome (Sct/Lss proteins): i.e., the basal body, the needle-like structure (*sctF*), and the translocator (*lopB*-, *lopD*-, and *lcrV*-like genes). As previously reported (28, 31), *Photorhabdus* TTSSs display many striking similarities to the *Yersinia pestis* and *Pseudomonas aeruginosa* TTSSs. *P. luminescens* subsp. *laumondii* and *P. luminescens* subsp. *akhurstii* as well as *P. asymbiotica* possess the same genetic organization required for the complete assembly of a functional TTSS machinery. Moreover, these three *Photorhabdus* species harbor genes similar to the *P. aeruginosa exsC* and *exsD* genes encoding transcriptional regulators, which are absent from *Yersinia* spp., suggesting that the regulation of this system is more related to that performing regulation in *P. aeruginosa*.

Second, the locations of the TTSS are identical in all these strains and are downstream of the *Enterobacteriaceae* housekeeping gene *cspI* encoding a cold shock protein (26). This finding suggests that prior to speciation, a *Photorhabdus* ancestor had acquired a TTSS as a block. As they are often plasmid encoded or located on pathogenicity islands, their mobile character has been suggested (29). In the case of the *Photorhabdus* TTSS, we did not detect the classical characteristics of a pathogenicity island (i.e., insertion into a tRNA gene and different GC content). However, four genes (*plu3747* through *plu3750*) encoding proteins similar to bacteriophage proteins were detected in the close vicinity of the *Photorhabdus*

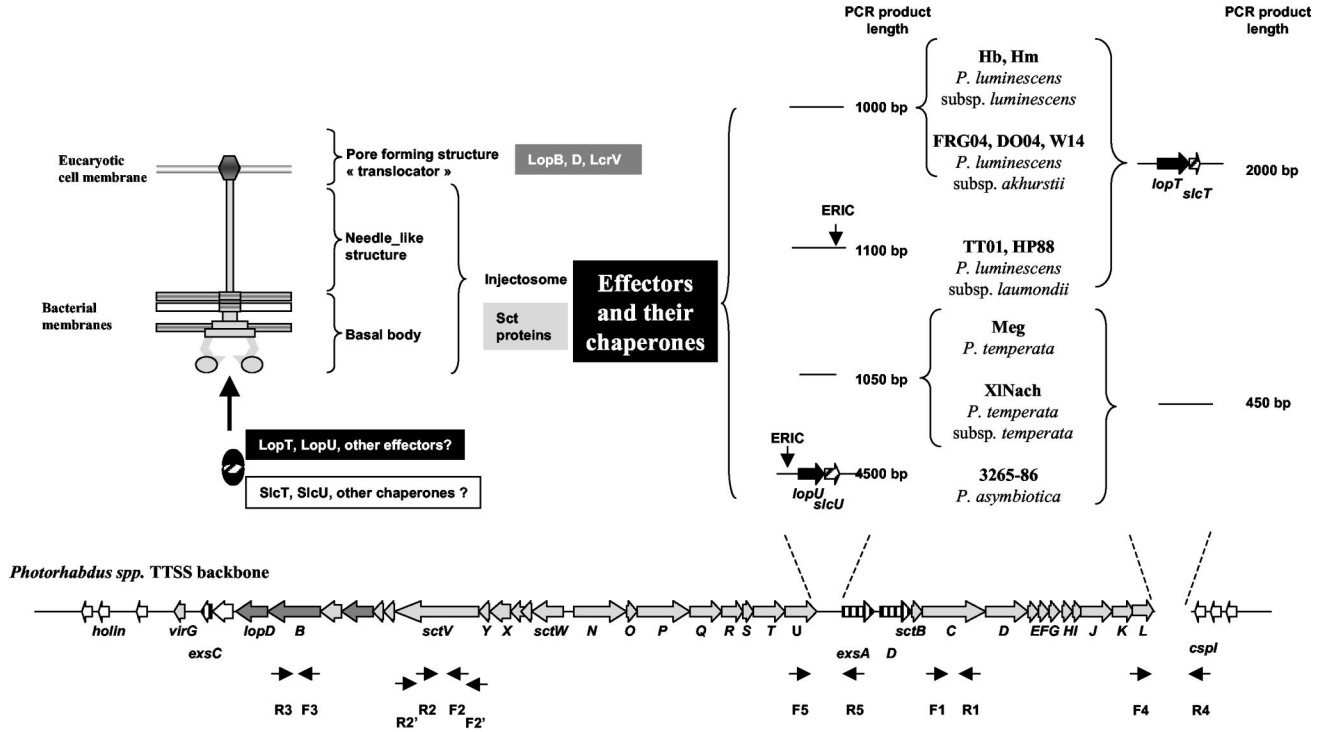

FIG. 1. Comparative genomics of TTSS organization in *Photorhabdus species*. Positions of the primer pairs are indicated. ERIC, enterobacterial repetitive intergenic consensus.

TTSS, suggesting that the *Photorhabdus* TTSS may have been acquired via an integrative bacteriophage.

Third, despite the highly conserved organization and protein sequences of the core components of the secretion machinery (TTSS backbone in Fig. 1), *P. luminescens* is predicted to encode a protein similar to the *Yersinia* YopT effector whereas *P. asymbiotica* harbors a gene encoding a protein homologous to the *P. aeruginosa* ExoU effector (see below).

Distribution of the TTSS among different strains of *Photorhabdus* and *Xenorhabdus* species. To determine whether all different *Photorhabdus* species and subspecies (Table 1) harbor similar TTSSs, PCR amplification was performed on genomic DNA of 11 *Photorhabdus* strains (Table 1). In addition, seven strains of the more distantly related *Xenorhabdus* species were included. The oligonucleotide primer sets used (Table 2) were designed in the conserved regions of the known TTSS sequences (Fig. 1). Genes of the delivery system were named according to the nomenclature proposed by Hueck (15). For this study, the *sctC*/*lssC*, *sctV*/*lssV*/*lcrD*, and *lopB* genes were chosen because they are representative of the different parts of the injectisome located in different operons (15). Standard PCR with each primer set was performed in a 50-μl reaction volume with a Gene Amp 2400 thermocycler system (Perkin Elmer), and PCR products were subjected to 0.7% agarose gel electrophoresis for analysis. Genomic DNA from the 11 *Photorhabdus* strains was successfully amplified using specific primers for these three genes (Table 3). However, amplification results were negative for the seven *Xenorhabdus* strains, even under lower-level annealing conditions.

As a second control for the presence of the TTSS, Southern blot analysis was performed using the *sctC*, *sctV*, and *lopB* genes as probes. *P. luminescens* TT01 genomic DNA was labeled using a PCR digoxigenin DNA labeling kit, and hybridization was revealed using a digoxigenin detection kit (Roche) according to the manufacturer's instructions. Southern blot hybridizations were performed on BglII,- EcoRI-, and HindIII-digested DNA from the previously mentioned *Photorhabdus* and *Xenorhabdus* strains (Table 1). No signal was detected using *Xenorhabdus* chromosomal DNA as a template even under low-stringency conditions, whereas a clear band of the expected size was visible when *Photorhabdus* chromosomal DNA was used as a template (data not shown). Thus, our results using PCR and Southern blotting data indicate that *sctC*, *sctV*, and *lopB* genes are conserved in all *Photorhabdus* strains. This is in agreement with a recent finding determined on the basis of a limited microarray analysis (18). Furthermore, our data suggest that species of the phylogenetically related genus *Xenorhabdus* (12) lack a TTSS or harbor a highly divergent system.

Phylogenetic analysis. To analyze the relatedness of the *Photorhabdus* TTSS and to compare it to the taxonomic position of the organism, a phylogenetic study was undertaken. We used the *sctV* gene (homologous to the *lcrD* gene of *Yersinia* spp.) encoding an inner-membrane protein, because *sctV* is among the best-conserved members of the TTSS. PCR products were isolated using a High Pure PCR purification product kit (Roche), and sequencing was performed on an ABI 3700 sequencer. Sequences were aligned using ClustalW (24), and phylogenetic trees were constructed by using the neighbor-joining method and Kimura distance values (21). A bootstrap confidence analysis was replicated 500 times (9).

The resulting trees are shown in Fig. 2. The various taxa

TABLE 1. Bacterial strains used in this study

Taxon and strain	Geographical origin	Host nematode or hospital strain	Accession number and/or source[a]
Photorhabdus luminescens subsp. *luminescens*			
Hb^T	South Australia	*Heterorhabditis bacteriophora*	CIP 106429
Hm	United States (Georgia)	*Heterorhabditis bacteriophora*	K. Nealson
Photorhabdus luminescens subsp. *akhurstii*			
FRG04^T	Guadeloupe	*Heterorhabditis indica*	CIP 105564
DO04	Dominican Republic	*Heterorhabditis indica*	L. Garrido
Photorhabdus luminescens subsp. *laumondii*			
TT01^T	Trinidad	*Heterorhabditis bacteriophora*	CIP 105565
HP88	United States	*Heterorhabditis bacteriophora*	R. Akhurst
Photorhabdus temperata			
Meg	United States	*Heterorhabditis megidis*	R. Akhurst
Photorhabdus temperata subsp. *temperata*			
X1Nach^T	Russia	*Heterorhabditis megidis*	CIP 105563
Photorhabdus asymbiotica subsp. *asymbiotica*			
3265-86^T	United States	Hospital strain[b]	CIP 106331
Photorhabdus spp.			
AU9800946	Australia	Hospital strain	R. Akhurst
AU9805888	Australia	Hospital strain	R. Akhurst
Xenorhabdus nematophila			
AN6^T	United States	*Steinernema carpocapsae*	ATCC1907
F1	France	*Steinernema carpocapsae*	C. Laumond
Xenorhabdus poinarii			
G6^T	United States	*Steinernema glaseri*	ATCC49121
Xenorhabdus beddingii			
Q58^T	Australia	*Steinernema* sp.	UQM2872
Xenorhabdus bovienii			
T228^T	Australia	*Steinernema feltiae*	UQM2211
Xenorhabdus japonica			
JP02^T	Japan	*Steinernema kushidai*	IAM14265
Xenorhabdus spp.			
USTX62	United States	*Steinernema riobrave*	E. Cabanillas

[a] CIP, strain collection of the Institut Pasteur; ATCC, American Type Culture Collection; UQM, culture collection of the University of Queensland-Australia, Brisbane, Queensland, Australia; IAM, Nippon culture collection of microorganisms.
[b] Centers for Disease Control and Prevention, Atlanta, Ga.

could be divided into five distinct groups (Fig. 2A): Ysc, Hrp1, Hrp2, Inv/Mxi/Spa, and EscC/Ssa (13); the *Photorhabdus* TTSS falls into the Ysc group. We extended this phylogenetic analysis to species of the genus *Photorhabdus* (Fig. 2B). Both clinical subspecies grouped in a single cluster. The three *P. luminescens* subspecies *P. luminescens* subsp. *laumondii*, *P. luminescens* subsp. *akhurstii*, and *P. luminescens* subsp. *luminescens* formed a second cluster, and the *P. temperata* strains formed a third cluster. Remarkably, the resulting *sctV* tree is similar to the 16S tree (1, 11), indicating that the TTSS phylogeny reflects the phylogeny of the group. Because the same clusters and subclusters describing genetic heterogeneity among strains belonging to the genus *Photorhabdus* can be distinguished, we therefore suggest that the *sctV* gene and probably the entire TTSS backbone were present in the *Photorhabdus* ancestor and were not recently independently acquired.

TABLE 2. Oligonucleotide primer pairs used

Target region	Oligonucleotide primer (5′-3′)
sctC	F1: AAT CAA CGC ACT GGA TAA AC R1: CAT CCT CAA TAT GCA GGT CT
sctV	F2: TTA ATT GGC GGT GTT TTA TT R2: AAT TCC AAC TGA CTG ACT GG
	F2′: GTG GTC TTC CAT GAT GGT TTT R2′: GAA TCG TAC GTG TAT TAC GA
lopB	F3: AGA ACC AGC AGA AGA TTA AAG R3: CAT CAC ATG TTG GAA AGA CTC
lopT-slcT	F4: CCT GAA ATC GCC TTA TTT AGA R4: TCA TTA TTC TGC AAT TCA GAG
lopU-slcU	F5: GCA GGC CCT ACA AGT TC R5: ATG TTG TGA TGC CGT TC

Analysis of the diversity of the *Photorhabdus* TTSS effectors. As the most remarkable elements are the genes predicted to encode the effectors, we therefore examined the two loci in which they lie in the 11 *Photorhabdus* strains previously mentioned. Oligonucleotide primers were selected in the flanking genes (i.e., *sctL* and *cspI* for the *lopT*/*slcT* locus and *sctU* and *lscA* for the *lopU*/*slcU* locus) and designed in conserved regions. PCR products were obtained for all 11 strains tested, and their sizes are reported in Table 3. PCR fragments were sequenced for strains Hb, DO04, Meg, 3265-86, and AU9800946, which are representatives of each subspecies. Sequences were aligned with the previously available sequences (i.e., those of strains TT01, W14, and *P. asymbiotica*).

TABLE 3. PCR assays for *sctC*, *sctV*, *lopB*, *lopT-spcT* and *lopU-spcU* in *Photorhabdus* and *Xenorhabdus* species

Species and strain	Amplicon size (kb) for primer (corresponding amplified region)[a]:				
	F1/R1 (*sctC*)	F2/R2 (*sctV*)	F3/R3 (*lopB*)	F4/R4 (*lopT-slcT*)	F5/R5 (*lopU-slcU*)
Photorhabdus luminescens subsp. *luminescens*					
Hb^T	0.5	**0.5**	0.8	2	1
Hm	0.5	**0.5**	0.8	2	1
P. luminescens subsp. *akhurstii*					
FRG04^T	0.5	**0.5**	0.8	2	1
DO04	0.5	1.5[b]	0.8	2	1
P. luminescens subsp. *laumondi*					
TT01^T	0.5	0.5	0.8	2	1.1
HP88	0.5	**0.5**	0.8	2	1.1
P. temperata					
Meg	0.5	**1.5**[b]	0.8	0.45	1.05
P. temperata subsp. *temperata*					
XINach^T	0.5	**0.5**	0.8	0.45	1
P. asymbiotica					
3265-86^T	0.5	**0.5**	0.8	0.45	4.5
Photorhabdus sp.					
AU9805888	0.5	**0.5**	0.8	0.45	—[c]
AU9800946	0.5	**0.5**	0.8	0.45	—
Xenorhabdus nematophila					
AN6T	—	—	—	—	—
F1	—	—	—	—	—
Xenorhabdus poinarii					
G6T	—	—	—	—	—
Xenorhabdus beddingii					
Q58T	—	—	—	—	—
Xenorhabdus bovienii					
T228T	—	—	—	—	—
Xenorahbdus japonica					
JP02T	—	—	—	—	—
Xenorhabdus sp.					
USTX62	—	—	—	—	—

[a] The presence of these genes was determined in this study using PCR assays. Amplicon sizes are reported. PCR products that were sequenced and used in phylogeny reconstructions (Fig. 2) or in comparisons (Fig. 1) are indicated in boldface characters.
[b] Amplification was performed using F2′/R2′ primers.
[c] —, no amplicon.

For the *sctL-cspI* locus, a 2,020-bp fragment containing the *lopT* and the *slcT* genes was amplified for all the *P. luminescens* strains and a 450-bp intergenic region was amplified for the *P. asymbiotica* and *P. temperata* strains. For the *sctU-lscA* region, we amplified a 4.5-kb fragment containing *lopU* and *slcU* genes for all the *P. asymbiotica* strains and a 1,000- to 1,100-bp intergenic region for *P. luminescens* and *P. temperata* strains. Intriguingly, no effector-encoding genes were detected in the DNA sequences at these locations in the *P. temperata* strains Meg and XlNach (Table 3 and Fig. 1); however, we could not rule out the possibility that they were elsewhere on the genome.

P. luminescens lopT is predicted to encode a protein similar to the *Yersinia* YopT effector. YopT is a cysteine protease (22, 23) that causes cytoskeletal disruption and contributes to the antiphagocytic effect of *Yersinia* (16, 30). The *P. luminescens lopT* gene is located at the 3′ end of the TTSS and, as in *Yersinia*, forms a bicistronic operon with a gene predicted to encode a LopT chaperone, namely, SlcT. These similarities with *Yersinia* Yop/SycT proteins are highly suggestive of a similar role for their *P. luminescens* counterpart, which is in agreement with the observation of LopT expression concomitant with the in vivo TTSS-dependent inhibition of phagocytosis in the orthopteran *Locusta migratoria* (Brugirard-Ricaud et al., submitted for publication).

Remarkably, the *P. asymbiotica* locus does not harbor *yopT* homologues. However, it contains *lopU*, a gene predicted to encode a protein similar to the *P. aeruginosa* ExoU effector. ExoU displays a potent phospholipase activity inducing disruption of epithelial and macrophage cell lines (10). The *P. asymbiotica lopU* gene is located between *sctU* and *exsA*-like genes and forms a probable operon with a gene predicted to encode a LopU chaperone, namely, SlcU. This location corresponds to the inversion point between the *Photorhabdus* and *P. aeruginosa* TTSS backbones (15).

In this report we show that all *Photorhabdus* species contain a remarkably conserved TTSS backbone but that the effectors seem to belong to the flexible gene pool, as they differ considerably among the different species. Moreover, enterobacterial repetitive intergenic consensus sequences were occasionally found in the vicinity of the effector loci, suggesting that they may be recombination hot spots and may account for genome plasticity. It is therefore tempting to speculate that in contrast to the TTSS backbone, the genes encoding the effectors may have been acquired at different steps of the evolution and then

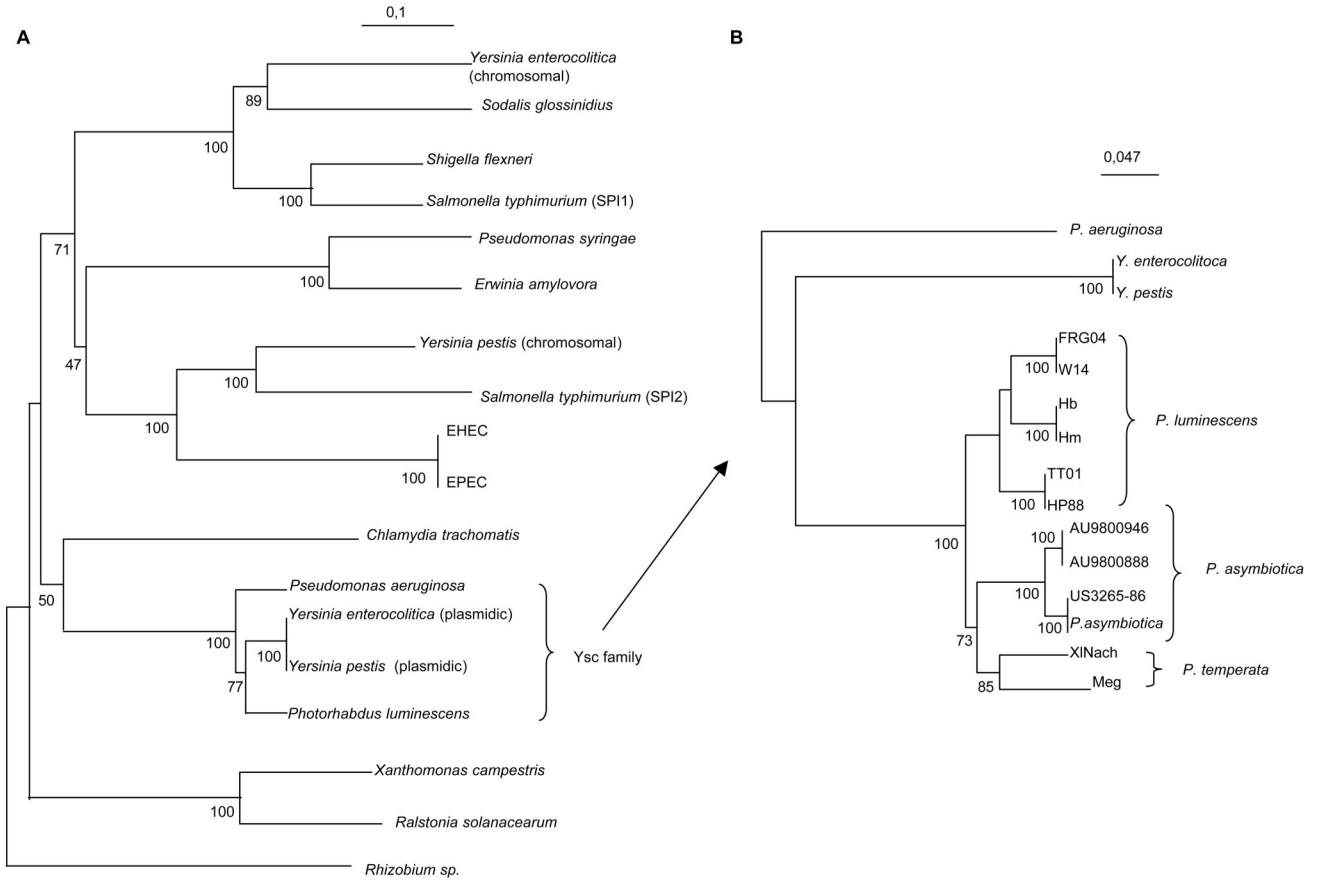

FIG. 2. Phylogenetic trees of bacterial *sctV* genes (A) and *Photorhabdus* species *sctV* genes (B). The ClustalW program with default parameters was used for aligning sequences and generating trees (branch strength values after bootstrapping 500 times are shown for some branches). Accession numbers of sequences used in this study are as follows: *Chlamydia trachomatis*, E0011283; *Erwinia amylovora*, P35654; *Escherichia coli* E2348/69 (enteropathogenic *E. coli* [EPEC]), AF022236; *E. coli* O157H7, NP_312596; *P. luminescens*, BX571871; *P. aeruginosa*, AF010150; *P. syringae*, P35655; *Ralstonia solanacearum*, P35656; *Rhizobium* sp. strain NGR 234, P55726; *Salmonella enterica* serovar Typhimurium (SPI1), P35657; *S. enterica* serovar Typhimurium (SPI2), P74856; *Shigella flexneri*, P35533; *Sodalis glossinidius*, AF306649; *Xanthomonas campestris*, P800150; *Y. enterocolitica* (chromosomal), AF369954; *Y. enterocolitica* (plasmid), P21210; *Y. pestis* (chromosomal), NC003143; *Y. pestis* (plasmid), P31487; strain Hb, AY526326; strain FRG04, AY526327; strain Hm, AY526331; strain HP88, AY526332; strain AU9800946, AY526330; strain AU9800888, AY526333; strain US3265-86, AY526329; strain XlNach, AY526328; strain Meg, AY526334.

have been selected according to the ecological niches and the host ranges of the different *Photorhabdus* species.

Nucleotide sequence accession number. The nucleotide sequences of *Photorhabdus* sp. strains Hb, Hm, FRG04, HP88, AU9800946, AU9800888, US3265-86, XlNach, and Meg were deposited in GenBank under accession no. AY526326, AY526331, AY526327, AY526332, AY526330, AY526333, AY656329, AY526328, and AY526334, respectively.

This work received financial support from the Institut National de la Recherche Agronomique and the Ministère de l'Industrie et des Finances (Après séquençage des Génomes). K.B.-R. was funded by a MENRT grant (2052.2001).

We wish to thank Isabelle Gonçalves for help with bioinformatics and Carmen Buchrieser for critical reading of the manuscript.

REFERENCES

1. **Akhurst, R. J., N. E. Boemare, P. H. Janssen, M. M. Peel, D. A. Alfredson, and C. E. Beard.** Taxonomy of Australian clinical isolates of the genus *Photorhabdus* and proposal of subspecies *Photorhabdus asymbiotica* subsp. *asymbiotica* subsp. nov. and *Photorhabdus asymbiotica* subsp. *australis* subsp. nov. Int. J. Syst. Evol. Microbiol., in press.
2. **Boemare, N., R. Akhurst, and R. Mourant.** 1993. DNA relatedness between *Xenorahbdus* spp. (*Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus luminescens* gen. nov. Int. J. Syst. Bacteriol. **43:**249–255.
3. **Cornelis, G. R., and F. Van Gijsegem.** 2000. Assembly and function of type III secretory systems. Annu. Rev. Microbiol. **54:**735–774.
4. **Dale, C., G. R. Plague, B. Wang, H. Ochman, and N. A. Moran.** 2002. Type III secretion systems and the evolution of mutualistic endosymbiosis. Proc. Natl. Acad. Sci. USA **99:**12397–12402.
5. **Dale, C., S. A. Young, D. T. Haydon, and S. C. Welburn.** 2001. The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. Proc. Natl. Acad. Sci. USA **98:**1883–1888.
6. **Donnenberg, M. S.** 2000. Pathogenic strategies of enteric bacteria. Nature **406:**768–774.
7. **Duchaud, E., C. Rusniok, L. Frangeul, C. Buchrieser, A. Givaudan, S. Taourit, S. Bocs, C. Boursaux-Eude, M. Chandler, J. F. Charles, E. Dassa, R. Derose, S. Derzelle, G. Freyssinet, S. Gaudriault, C. Medigue, A. Lanois, K. Powell, P. Siguier, R. Vincent, V. Wingate, M. Zouine, P. Glaser, N. Boemare, A. Danchin, and F. Kunst.** 2003. The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. Nat. Biotechnol. **21:**1307–1313.
8. **Farmer, J. J., III, J. H. Jorgensen, P. A. Grimont, R. J. Akhurst, G. O. Poinar, Jr., E. Ageron, G. V. Pierce, J. A. Smith, G. P. Carter, K. L. Wilson, et al.** 1989. *Xenorhabdus luminescens* (DNA hybridization group 5) from human clinical specimens. J. Clin. Microbiol. **27:**1594–1600.
9. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.

10. **Finck-Barbancon, V., J. Goranson, L. Zhu, T. Sawa, J. P. Wiener-Kronish, S. M. Fleiszig, C. Wu, L. Mende-Mueller, and D. W. Frank.** 1997. ExoU expression by *Pseudomonas aeruginosa* correlates with acute cytotoxicity and epithelial injury. Mol. Microbiol. **25:**547–557.
11. **Fischer-Le Saux, M., V. Viallard, B. Brunel, P. Normand, and N. E. Boemare.** 1999. 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. Int. J. Syst. Bacteriol. **49:**1645–1656.
12. **Forst, S., B. Dowds, N. Boemare, and E. Stackebrandt.** 1997. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annu. Rev. Microbiol. **51:**47–72.
13. **Foultier, B., P. Troisfontaines, S. Muller, F. R. Opperdoes, and G. R. Cornelis.** 2002. Characterization of the *ysa* pathogenicity locus in the chromosome of *Yersinia enterocolitica* and phylogeny analysis of type III secretion systems. J. Mol. Evol. **55:**37–51.
14. **Hacker, J., G. Blum-Oehler, I. Muhldorfer, and H. Tschape.** 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23:**1089–1097.
15. **Hueck, C. J.** 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol. Mol. Biol. Rev. **62:**379–433.
16. **Iriarte, M., and G. R. Cornelis.** 1998. YopT, a new *Yersinia* Yop effector protein, affects the cytoskeleton of host cells. Mol. Microbiol. **29:**915–929.
17. **Lee, C. A.** 1996. Pathogenicity islands and the evolution of bacterial pathogens. Infect. Agents Dis. **5:**1–7.
18. **Marokhazi, J., N. Waterfield, G. LeGoff, E. Feil, R. Stabler, J. Hinds, A. Fodor, and R. H. ffrench-Constant.** 2003. Using a DNA microarray to investigate the distribution of insect virulence factors in strains of *Photorhabdus* bacteria. J. Bacteriol. **185:**4648–4656.
19. **Peel, M. M., D. A. Alfredson, J. G. Gerrard, J. M. Davis, J. M. Robson, R. J. McDougall, B. L. Scullie, and R. J. Akhurst.** 1999. Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. J. Clin. Microbiol. **37:**3647–3653.
20. **Poulin, R., and C. Combes.** 1999. The concept of virulence: interpretations and implications. Parasitol. Today **15:**474–475.
21. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
22. **Shao, F., and J. E. Dixon.** 2003. YopT is a cysteine protease cleaving Rho family GTPases. Adv. Exp. Med. Biol. **529:**79–84.
23. **Shao, F., P. M. Merritt, Z. Bao, R. W. Innes, and J. E. Dixon.** 2002. A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. Cell **109:**575–588.
24. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
25. **Viprey, V., A. Del Greco, W. Golinowski, W. J. Broughton, and X. Perret.** 1998. Symbiotic implications of type III protein secretion machinery in *Rhizobium*. Mol. Microbiol. **28:**1381–1389.
26. **Wang, N., K. Yamanaka, and M. Inouye.** 1999. CspI, the ninth member of the CspA family of *Escherichia coli*, is induced upon cold shock. J. Bacteriol. **181:**1603–1609.
27. **Wassenaar, T. M., and W. Gaastra.** 2001. Bacterial virulence: can we draw the line? FEMS Microbiol. Lett. **201:**1–7.
28. **Waterfield, N. R., P. J. Daborn, and R. H. ffrench-Constant.** 2002. Genomic islands in *Photorhabdus*. Trends Microbiol. **10:**541–545.
29. **Winstanley, C., and C. A. Hart.** 2001. Type III secretion systems and pathogenicity islands. J. Med. Microbiol. **50:**116–126.
30. **Zumbihl, R., M. Aepfelbacher, A. Andor, C. A. Jacobi, K. Ruckdeschel, B. Rouot, and J. Heesemann.** 1999. The cytotoxin YopT of *Yersinia enterocolitica* induces modification and cellular redistribution of the small GTP-binding protein RhoA. J. Biol. Chem. **274:**29289–29293.
31. **Zumbihl, R., A. Lanois, K. Brugirard-Ricaud, J. Brillard, S. Pagès, and A. Givaudan.** 2002. *Xenorhabdus* and *Photorhabdus* virulence factor and their impacts on insect cellular immunity, p. 177–183. Proceedings of the XXXV Annual Meeting of the Society for Invertebrate Pathology, Iguassu Fall, Brazil.