New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species

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We investigated the diversity of a collection of 76 *Xenorhabdus* strains, isolated from at least 27 species of *Steinernema* nematodes and collected in 32 countries, using three complementary approaches: 16S rRNA gene sequencing, molecular typing and phenotypic characterization. The 16S rRNA gene sequences of the *Xenorhabdus* strains were highly conserved (similarity coefficient > 95 %), suggesting that the common ancestor of the genus probably emerged between 250 and 500 million years ago. Based on comparisons of the 16S rRNA gene sequences, we identified 13 groups and seven unique sequences. This classification was confirmed by analysis of molecular typing profiles of the strains, leading to the classification of new isolates into the *Xenorhabdus* species described previously and the description of ten novel *Xenorhabdus* species: *Xenorhabdus cabanillasii* sp. nov. (type strain USTX62^T = CIP 109066^T = DSM 17905^T), *Xenorhabdus doucetiae* sp. nov. (type strain FRM16^T = CIP 109074^T = DSM 17909^T), *Xenorhabdus griffiniae* sp. nov. (type strain ID10^T = CIP 109073^T = DSM 17911^T), *Xenorhabdus hominickii* sp. nov. (type strain KE01^T = CIP 109072^T = DSM 17903^T), *Xenorhabdus koppenhoeferi* sp. nov. (type strain USNJ01^T = CIP 109199^T = DSM 18168^T), *Xenorhabdus kozodoii* sp. nov. (type strain SaV^T = CIP 109068^T = DSM 17907^T), *Xenorhabdus mauleonii* sp. nov. (type strain VC01^T = CIP 109075^T = DSM 17908^T), *Xenorhabdus miraniensis* sp. nov. (type strain Q1^T = CIP 109069^T = DSM 17902^T), *Xenorhabdus romanii* sp. nov. (type strain PR06-A^T = CIP 109070^T = DSM 17910^T) and *Xenorhabdus stockiae* sp. nov. (type strain TH01^T = CIP 109067^T = DSM 17904^T). The *Xenorhabdus* strains studied here had very similar phenotypic patterns, but phenotypic features nonetheless differentiated the following species: *X. bovienii*, *X. cabanillasii*, *X. hominickii*, *X. kozodoii*, *X. nematophila*, *X. poinarii* and *X. szentirmaii*. Based on phenotypic analysis, we identified two major groups of strains. Phenotypic group G_A comprised strains able to grow at temperatures of 35–42 °C, whereas phenotypic group G_B comprised strains that grew at temperatures below 35 °C, suggesting that some *Xenorhabdus* species may be adapted to tropical or temperate regions and/or influenced by the growth and development temperature of their nematode host.

The genus *Xenorhabdus* was first described by Thomas & Poinar (1979). The bacteria of this genus are symbiotically associated with insect-pathogenic nematodes of the genus *Steinernema* (Travassos, 1927). This genus currently comprises *Xenorhabdus nematophila* Thomas and Poinar 1979 (the type species), three further species described by Akhurst & Boemare (1988) (*Xenorhabdus beddingii*, *X. bovienii* and *X. poinarii*), *Xenorhabdus japonica* described by Nishimura *et al.* (1994), four species described recently by Lengyel *et al.* (2005) (*Xenorhabdus budapestensis*, *X. ehlersii*, *X. innexi*, *X. szentirmaii*) and '*Xenorhabdus indica*',

described by Somvanshi *et al.* (2006) but not yet validly published. Our collection comprised 76 *Xenorhabdus* strains isolated from at least 27 *Steinernema* species collected in 32 countries. The strains were characterized by molecular and phenotypic approaches. The phylogenetic spectrum of these *Xenorhabdus* strains was found to be broad, including many novel species dispersed among type strains in the 16S rRNA gene phylogenetic tree.

Bacterial strains and culture conditions

The bacterial strains studied were part of our collection (31 strains) (Université Montpellier II, France) or were provided by the DSMZ collection (six strains) (<http://>

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences obtained in this study are listed in Table 1.

www.dsmz.de) (Table 1). Thirty-nine new isolates were obtained from the infective stages of nematodes of the genus *Steinernema* (Table 1) by the hanging-drop technique (Poinar, 1966). Bacteriological purity was checked by plating on nutrient agar supplemented with 0.004 % (w/v) triphenyltetrazolium chloride and 0.0025 % (w/v) bromothymol blue (NBTA medium) at 28 °C (Akhurst, 1980). The isolates were examined for the main phenotypic characteristics of the genus *Xenorhabdus*, using the methods of Boemare & Akhurst (1988). Strains were stored at -80 °C in LB broth (Difco) containing 15 % glycerol (v/v).

Phenotypic characterization of bacterial strains and data analysis

Growth at various temperatures from 32 to 42 °C, enzymic activities on API 20 E and API 20 NE strips (bioMérieux), carbon source assimilation on Biotype 100 (bioMérieux) and fermentation on API 50 CH strips (bioMérieux) were assessed as described by Fischer-Le Saux *et al.* (1999b). Phenotypic tests on agar plates (ampicillin susceptibility, dye adsorption, colony pigmentation, antibiosis, cultures in Tween media, gelatin hydrolysis, DNase and lecithinase activities, haemolysis) have been described elsewhere (Akhurst, 1980; Boemare & Akhurst, 1988; Akhurst *et al.*, 1996). Phenotypic data were analysed with BioNumerics software (Applied Maths). Five similarity matrices were calculated using only variable characters, each corresponding to a set of data: growth at different temperatures (11 variable characters), enzyme activities ($n=10$), carbon source assimilation ($n=69$), carbon source fermentation ($n=45$) and phenotypic tests on agar plates ($n=18$). Weakly positive results were scored as 0.5 and the data were compared using the Gower similarity coefficient (Gower & Legendre, 1986). The five similarity matrices were combined such that each set of data contributed equally to the combined similarity matrix, regardless of the number of characters it contained. The resulting dendrogram was calculated by the unweighted pair group method using arithmetic means (UPGMA) (Sokal & Michener, 1958).

Molecular characterization of bacterial strains and data analysis

Total bacterial genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen). The almost-complete 16S rRNA gene was amplified by PCR using primers 16SP1 (5'-GAAGAGTTTGATCATGGCTC-3', corresponding to *Escherichia coli* 16S rRNA positions 6–25, forward) and 16SP2 (5'-AAGGAGGTGATCCAGCCGCA-3', corresponding to positions 1522–1540, reverse). PCR was carried out in a final volume of 50 µl containing 20–100 ng DNA, 3 mM MgCl₂ (Invitrogen), 0.1 mM of each primer, 200 µM of each dNTP (Invitrogen) and 2.5 U *Taq* DNA polymerase (Invitrogen) in the buffer supplied with the enzyme. Amplification conditions were: 94 °C for 2 min followed by 35 cycles of 30 s at 95 °C, 30 s at 63 °C and 1 min at 72 °C, followed by 7 min at 72 °C. PCR products were purified using a Montage PCR device (Millipore). Sequences

overlapping the 16S rRNA gene were then obtained using three sequencing primers (SP1, 5'-ACCGCGGCTGCTGGCAG-3', position 514 reverse; SP2, 5'-CTCGTTGCGGGACTTAAC-3', position 1089 reverse; and 16SP2) and merged using SeqMan II (DNASTar). Multiple alignments of the 16S rRNA gene sequences were obtained with CLUSTAL W (<http://clustalw.genome.jp/>) and the distance tree was calculated using the model of Jukes & Cantor (1969) and the neighbour-joining (NJ) method (Saitou & Nei, 1987) included in PAUP software (Swofford, 2003). Bootstrap analysis was carried out with 1000 datasets. We also used the maximum-likelihood (ML) and parsimony methods included in PAUP to compare the topologies of the phylogenetic trees obtained for a given set of sequences.

The 16S rRNA gene restriction fragment length polymorphism (RFLP) typing method was carried out as described previously (Fischer-Le Saux *et al.*, 1998). The RFLP data are not presented here, but were used to select representative strains of *X. bovienii* (11/25), *X. nematophila* (6/13) and *X. poinarii* (4/5) from the strains of our collection for which 16S rRNA gene sequences have been determined. Randomly amplified polymorphic DNA (RAPD) profiles (Williams *et al.*, 1990) were determined using primers P1 (5'-TGCTCTGCCC-3'), P2 (5'-GGTGACGCAG-3') and P3 (5'-TCGCTGGGAC-3') in separate reactions. Enterobacterial repetitive intergenic consensus (ERIC) (Hulton *et al.*, 1991) PCR profiles were determined using the primers ERIC1R (5'-GCTATGCTCCYGGGRTT-3') and ERIC2 (5'-ACTATGTGAYTGGGGTGA-3'). The sequences of the ERIC primers proposed by Versalovic *et al.* (1991) were modified to correspond to the ERIC sequences present in the *Xenorhabdus* genome (http://maizeapache.ddpsc.org/xeno_blast/index.html). PCR amplifications were performed in a final volume of 50 µl containing 1 × PCR buffer (Qbiogene), 20–100 ng bacterial genomic DNA, 0.2 mM MgCl₂, 0.5 µM primer, 200 µM of each dNTP and 3.75 U *Taq* DNA polymerase (concentration of enzyme stock 15 U µl⁻¹). PCR was carried out in a GeneAmp 2400 thermal cycler PCR system (Perkin Elmer) programmed for 30 cycles of amplification of 1 min at 94 °C, 1 min at 48 °C (ERIC) or 42 °C (RAPD), 3 min (ERIC) to 6 min (RAPD) of temperature ramping to 72 °C and 1 min at 72 °C, after an initial 5 min denaturation at 94 °C. Electrophoresis was carried out as described previously (Tailliez *et al.*, 1998). Molecular typing profiles were acquired with a CCD camera (Sony) and photocapture software (PHOTO-CAPT; Fisher Bioblock Scientific). For each strain, the ERIC profile and the three RAPD profiles were combined and then compared using Pearson's similarity coefficient. The resulting dendrogram was calculated with the UPGMA module of the GelCompar software (Applied-Maths).

Comparison of 16S rRNA gene sequences

Fig. 1 shows the distance tree resulting from comparison of the 16S rRNA gene sequences of 54 representative *Xenorhabdus* strains selected from the 76 strains studied (Table 1). Representative strains were selected from the

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

Type strains and proposed type strains are highlighted in bold.

Strain	Previously classified as:	Proposed classification	16S rRNA gene GenBank accession no.	Group	<i>Steinernema</i> host species	Geographical origin	Source	Reference(s)*
Strains assigned to recognized species								
Q58^T	<i>X. beddingii</i>	<i>X. beddingii</i>	AY278675		Unknown	Australia	R. Akhurst	2, 3, 4, 5, 6, 11
T228^T	<i>X. bovienii</i>	<i>X. bovienii</i>	X82252	G ₈	<i>S. feltiae</i>	Australia	R. Akhurst	1, 4, 5, 6
Dan	<i>X. bovienii</i>	<i>X. bovienii</i>		G ₈	<i>S. affine</i>	Denmark	R. Akhurst	3
F3	<i>X. bovienii</i>	<i>X. bovienii</i>	DQ202311	G ₈	<i>S. affine</i>	France	E. Bonifassi	3, 4, 5, 6
F5	<i>X. bovienii</i>	<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	France	E. Bonifassi	3, 5, 6
F7	<i>X. bovienii</i>	<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	France	E. Bonifassi	3
FR10	<i>X. bovienii</i>	<i>X. bovienii</i>	AY521240	G ₈	<i>S. feltiae</i>	France	E. Bonifassi	11
N60	<i>X. bovienii</i>	<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	Australia	R. Akhurst	3
Si	<i>X. bovienii</i>	<i>X. bovienii</i>	DQ205455	G ₈	<i>S. intermedium</i>	USA	R. Akhurst	4, 5, 6
SK2	<i>X. bovienii</i>	<i>X. bovienii</i>		G ₈	<i>S. kraussei</i>	Czech Republic	R. Akhurst	3, 4, 5, 6
BE04		<i>X. bovienii</i>		G ₈	<i>S. affine</i>	Belgium	M. Moens	This work
BE05		<i>X. bovienii</i>		G ₈	<i>S. affine</i>	Belgium	M. Moens	This work
CA04		<i>X. bovienii</i>	DQ205454	G ₈	<i>S. kraussei</i>	Canada	G. Bélair	This work
CS03		<i>X. bovienii</i>	DQ205452	G ₈	<i>S. weiseri</i>	Czech Republic	Z. Mráček	This work
CS66		<i>X. bovienii</i>	DQ205451	G ₈	<i>S. kraussei</i>	Czech Republic	Z. Mráček	This work
FR11		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	France	E. Bonifassi	This work
FR12		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	France	E. Bonifassi	This work
USCA99		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	USA	P. Stock	This work
SN1		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	France	P. Stock	This work
TB01		<i>X. bovienii</i>		G ₈	Not yet defined	China	Z. Mráček	This work
TB10		<i>X. bovienii</i>	DQ208304	G ₈	Not yet defined	China	Z. Mráček	This work
TB20		<i>Xenorhabdus</i> sp.	DQ208305	G ₈	Not yet defined	China	Z. Mráček	This work
TB30		<i>X. bovienii</i>	DQ208306	G ₈	Not yet defined	China	Z. Mráček	This work
TR15		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	Turkey	P. Stock	This work
USFL53		<i>X. bovienii</i>		G ₈	<i>S. feltiae</i>	USA	G. Smart	This work
USNY95		<i>X. bovienii</i>	DQ205453	G ₈	<i>S. kraussei</i>	USA	P. Stock	This work
DSM 16342^T	<i>X. budapestensis</i>	<i>X. budapestensis</i>	AJ810293	G ₂	<i>S. bicornutum</i>	Serbia	DSMZ	8
CN03		<i>X. budapestensis</i>	DQ211714	G ₂	<i>S. ceratophorum</i>	China	P. Stock	This work
DSM 16337^T	<i>X. ehlersii</i>	<i>X. ehlersii</i>	AJ810294	G ₁	<i>S. serratum</i> †	China	DSMZ	8
CN01		<i>X. ehlersii</i>	DQ208307	G ₁	<i>S. serratum</i> †	China	J. Liu	6
KR02		<i>X. ehlersii</i>	DQ208308	G ₁	<i>S. longicaudum</i>	Korea	H. Choo	This work
KR03		<i>X. ehlersii</i>	DQ202306	G ₁	Unknown	Korea	H. Choo	This work
USCA98		<i>X. ehlersii</i>	DQ202312	G ₁	<i>S. longicaudum</i>	USA	P. Stock	This work
DSM 17382	‘ <i>X. indica</i> ’	‘ <i>X. indica</i> ’	AM040494	G ₅	<i>S. thermophilum</i>	India	DSMZ	12
OM01		‘ <i>X. indica</i> ’	DQ211718	G ₅	<i>S. abbasi</i>	Sultanate of Oman	S. Elawad	This work

Table 1. cont.

Strain	Previously classified as:	Proposed classification	16S rRNA gene GenBank accession no.	Group	<i>Steinernema</i> host species	Geographical origin	Source	Reference(s)*
DSM 16336^T	<i>X. innexi</i>	<i>X. innexi</i>	AJ810292	G ₃	<i>S. scapterisci</i>	Uruguay	DSMZ	8
UY61		<i>X. innexi</i>	AY521243	G ₃	<i>S. scapterisci</i>	Uruguay	G. Smart	6, 11
DSM 16522^T	<i>X. japonica</i>	<i>X. japonica</i>	DQ202310	G ₆	<i>S. kushidai</i>	Japan	DSMZ	9
AN6^{T‡}	<i>X. nematophila</i>	<i>X. nematophila</i>	AY278674	G ₁₀	<i>S. carpocapsae</i>	USA	R. Akhurst	4, 5, 6, 11
A24	<i>X. nematophila</i>	<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	Russia	R. Akhurst	1, 3, 4, 5, 6
DD136‡	<i>X. nematophila</i>	<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	USA	C. Laumond	10
F1	<i>X. nematophila</i>	<i>X. nematophila</i>	AY521241	G ₁₀	<i>S. carpocapsae</i>	France	E. Bonifassi	3, 4, 5, 6, 11
NC116	<i>X. nematophila</i>	<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	USA	R. Akhurst	3
PL31	<i>X. nematophila</i>	<i>X. nematophila</i>	AY521242	G ₁₀	<i>S. carpocapsae</i>	Poland	J. Jarosz	11
A20		<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	Portugal	N. Simões	This work
BE06		<i>X. nematophila</i>	DQ211704	G ₁₀	<i>S. carpocapsae</i>	Belgium	M. Moens	This work
CA01		<i>X. nematophila</i>	DQ211705	G ₁₀	<i>S. carpocapsae</i>	Canada	G. Bélair	This work
CBY		<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	China	R. Akhurst	This work
ES96		<i>X. nematophila</i>	DQ211707	G ₁₀	<i>S. carpocapsae</i>	Spain	R. Campos Herrera	This work
USCA97		<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	USA	G. Poinar	This work
MX102		<i>X. nematophila</i>		G ₁₀	<i>S. carpocapsae</i>	Mexico	G. Poinar	This work
G6^T	<i>X. poinarii</i>	<i>X. poinarii</i>	D78010	G ₉	<i>S. glaseri</i>	USA	R. Akhurst	4, 5, 6, 7, 11
CU01	<i>X. poinarii</i>	<i>X. poinarii</i>	DQ211706	G ₉	<i>S. cubanum</i>	Cuba	E. Arteaga	7
NC33	<i>X. poinarii</i>	<i>X. poinarii</i>		G ₉	<i>S. glaseri</i>	USA	R. Akhurst	3, 5, 6, 7
SK72	<i>X. poinarii</i>	<i>X. poinarii</i>	AY521239	G ₉	<i>S. glaseri</i>	USA	N. Simões	6, 7, 11
AZ26		<i>X. poinarii</i>	DQ211703	G ₉	<i>S. glaseri</i>	Portugal	N. Simões	This work
DSM 16338^T	<i>X. szentirmaii</i>	<i>X. szentirmaii</i>	AJ810295	G ₁₂	<i>S. rarum</i>	Argentina	DSMZ	8
AR81		<i>X. szentirmaii</i>	DQ211708	G ₁₂	<i>S. rarum</i>	Argentina	P. Stock	This work
K77		<i>X. szentirmaii</i>	DQ211712	G ₁₂	<i>S. rarum</i>	Argentina	M. Doucet	4, 5, 6
Strains assigned to novel species								
KE01^T		<i>X. hominickii</i>	DQ211719	G ₇	<i>S. kari</i>	Kenya	P. Stock	This work
KR01		<i>X. hominickii</i>	DQ205448	G ₇	<i>S. monticolum</i>	Korea	P. Stock	6
KR05		<i>X. hominickii</i>	DQ205449	G ₇	<i>S. monticolum</i>	Korea	P. Stock	This work
SaV^T		<i>X. kozodoii</i>	DQ211716	G ₄	<i>S. arenarium</i>	Russia	R. Akhurst	4, 5, 6
ES01		<i>X. kozodoii</i>	DQ202308	G ₄	Not yet defined	Spain	O. Triggiani	This work
IT10		<i>X. kozodoii</i>	DQ202308	G ₄	<i>S. apuliae</i>	Italia	O. Triggiani	This work
FRM16^T		<i>X. doucetiae</i>	DQ211709	G ₁₁	<i>S. diaprepesi</i>	Martinique	H. Mauléon	6
FRG30		<i>X. doucetiae</i>	DQ211702	G ₁₁	<i>S. diaprepesi</i>	Guadeloupe	H. Mauléon	This work
USTX62^T		<i>X. cabanillasii</i>	AY521244	G ₁₃	<i>S. riobrave</i>	USA	E. Cabanillas	6, 11
JM26		<i>X. cabanillasii</i>	DQ211711	G ₁₃	<i>S. riobrave</i>	Jamaica	H. Mauléon	6
ID10^T		<i>X. griffiniae</i>	DQ211710		<i>S. hermaphroditum</i>	Indonesia	C. Griffin	This work
PR06-A^T		<i>X. romani</i>	DQ211717		<i>S. puertoricense</i>	Puerto Rico	H. Mauléon	6
Q1^T		<i>X. miraniensis</i>	DQ211713		Steinernematidae	Australia	R. Akhurst	4
TH01^T		<i>X. stockiae</i>	DQ202309		<i>S. siamkayai</i>	Thailand	P. Stock	This work

Table 1. cont.

Strain	Previously classified as:	Proposed classification	16S rRNA gene GenBank accession no.	Group	<i>Steinernema</i> host species	Geographical origin	Source	Reference(s)*
USNJ01 ^T		<i>X. koppenhoeferi</i>	DQ205450		<i>S. scarabaei</i>	USA	A. Koppenhöfer	This work
VC01 ^T		<i>X. maulenonii</i>	DQ211715		Unknown	St. Vincent	H. Mauléon	6
VN01		<i>Xenorhabdus</i> sp.	DQ205447	G ₆	<i>S. sangi</i>	Vietnam	P. Stock	This work

*References: 1, Akhurst (1983); 2, Akhurst (1986); 3, Akhurst & Boemare (1988); 4, Boemare *et al.* (1993); 5, Brunel *et al.* (1997); 6, Fischer-Le Saux *et al.* (1998); 7, Fischer-Le Saux *et al.* (1999a); 8, Lengyel *et al.* (2005); 9, Nishimura *et al.* (1994); 10, Poinar & Thomas (1965); 11, Sicard *et al.* (2004); 12, Somvanshi *et al.* (2006).

†Strains CN01 and DSM 16337^T were provided as symbiotic bacteria of the nematode species *S. serratum*. However, the *Steinernema* strain described as *S. serratum* was in fact a strain of *S. longicaudum*. The designation *S. serratum* should therefore be considered invalid (Qiu *et al.*, 2004).

‡*X. nematophila* DD136 and *X. nematophila* AN6^T from our collection, which initially originated from *X. nematophila* DD136 (*Achromobacter nematophilus*) isolated by Dutky & Hough (1955), studied by Poinar & Thomas (1965) and deposited at the ATCC under accession number ATCC 19061^T, were distinguished here on the basis of molecular typing profiles.

species *X. nematophila*, *X. bovienii* and *X. poinarii*, taking into account geographical origin, species of nematode host and the diversity of 16S rRNA gene RFLP profiles. The 16S rRNA gene sequence obtained from strain IAM 14265^T, which should correspond to *X. japonica* SK-1^T, was actually similar to that of *X. nematophila* strains and therefore significantly different from the sequence of *X. japonica* SK-1^T deposited in GenBank under accession number D78008. Our result was confirmed by the staff of the IAM collection. We therefore used the *X. japonica* type strain deposited in the DSMZ under accession number DSM 16522^T instead. This strain has a 16S rRNA gene sequence (GenBank accession number DQ202310) very similar to the GenBank sequence of *X. japonica* SK-1^T.

The 16S rRNA gene sequences of the *Xenorhabdus* strains studied here displayed more than 95 % similarity. If we consider a mean substitution rate for the 16S rRNA in eubacteria of about 1–2 % per 50 million years (Ochman & Wilson, 1987; Moran *et al.*, 1993), the common ancestor of the genus *Xenorhabdus* probably emerged between 250 and 500 million years ago. These data are consistent with Poinar's speculations that nematodes of the genus *Steinernema* probably emerged about 375 million years ago (Poinar, 1993). On the bacterial evolution time scale, we can consider the genus *Xenorhabdus* to have emerged relatively recently.

Forty-seven sequences were distributed between 13 groups, G₁ to G₁₃, based on high bootstrap values (>98 %) in the distance tree. Seven sequences, corresponding to strains TH01^T, ID10^T, USNJ01^T, VC01^T, Q1^T, PR06-A^T and *X. beddingii* DSM 4764^T, were not included in these groups. The composition of the 13 groups of sequences was confirmed using the ML and parsimony methods of phylogenetic tree reconstruction and using the 16S rRNA gene sequences of *Photorhabdus luminescens* subsp. *laumondii* TT01^T (GenBank accession no. AJ007404), *Proteus vulgaris* CIP 103181^T (AJ301683) and *E. coli* ATCC 11775^T (X80725) as outgroups (data not shown). Although the bootstrap values at the nodes between groups G₁ to G₄ were less than 50 %, these four groups were linked similarly by the ML and parsimony methods, suggesting that they were phylogenetically related. Similarly, the sequence of strain ID10^T was linked to those of group G₅ and the sequence of strain PR06-A^T was linked to those of group G₁₁ in all three methods of phylogenetic tree reconstruction. The sequences of strains TH01^T, USNJ01^T, VC01^T and Q1^T and the *X. beddingii* type strain DSM 4764^T were not robustly linked to any of the 13 groups of sequences defined in this work. Moreover, relationships between the groups, with the exception of relationships between G₁, G₂, G₃ and G₄ (see above), appeared to be unreliable, suggesting (i) that the 16S rRNA gene sequences used were insufficiently informative for phylogenetic analysis and/or (ii) an explosive radiation of diversity within the genus *Xenorhabdus*.

Comparison of molecular typing profiles

The differences between two *Xenorhabdus* 16S rRNA gene sequences were often less than 3 % (and always less than

Fig. 1. Distance tree showing the phylogenetic relationships of 54 *Xenorhabdus* strains and isolates. The tree was constructed using 16S rRNA gene sequences (1433 nucleotides), the model of Jukes & Cantor (1969) and the neighbour-joining module of PAUP software. *Photorhabdus luminescens* subsp. *laumondii* TT01^T was used as an outgroup. Bootstrap values (percentages of 1000 replicates) of more than 90% are shown at nodes. Dashed lines indicate unreliable links between groups and unique sequences that were not confirmed by the ML and parsimony methods of phylogenetic tree reconstruction. The underlined accession numbers in parentheses correspond to 16S rRNA gene sequences retrieved from GenBank; the other sequences were determined in this study. Thirteen groups, G₁ to G₁₃, supported by high bootstrap values (>98%), were identified. Sequences corresponding to type strains are highlighted in bold. When known, the *Steinernema* species of the corresponding nematode host is indicated. Bar, 1% sequence divergence.

5%), so this frequently used bacterial taxonomy threshold (Stackebrandt & Goebel, 1994) cannot be used here to support proposals for novel species of *Xenorhabdus*. This observation was also true for the genus *Photorhabdus* of nematode-symbiotic bacteria (Akhurst *et al.*, 2004). Quantitative DNA–DNA hybridization data were available only for representative strains of five species with validly published names, *X. nematophila*, *X. bovienii*, *X. poinarii*, *X. beddingii* and *X. japonica* (Boemare *et al.*, 1993; Nishimura *et al.*, 1994), and for strains K77, SaV^T and Q1^T (Boemare *et al.*, 1993). We therefore assessed the relationships between our strains using a combination of molecular typing techniques (ERIC and RAPD fingerprinting) that could be performed easily for such a large collection of bacteria. These molecular typing methods are based on the amplification of conserved and variable regions of the genome. They enabled us to group together strains belonging to the same species based on the amplification of conserved genomic regions (migration of bands of the same size in different profiles). This approach was used successfully for *Campylobacter* and its relatives (Mazurier *et al.*, 1992), for which phenotypic

inertness has prevented the development of a phenotypic identification scheme (Vandamme *et al.*, 1996), and for some closely related species of lactobacilli showing an overall phenotypic similarity (e.g. the *Lactobacillus acidophilus* group) (Gancheva *et al.*, 1999). Similarly, the combined use of RAPD and ERIC fingerprinting was used successfully to differentiate *Salmonella* species (Lim *et al.*, 2005).

A comparison of the molecular typing profiles of the 76 *Xenorhabdus* strains studied (Fig. 2) led to the identification of groups corresponding to those defined by 16S rRNA gene sequences analysis, except for group G₆, in which strain VN01 and *X. japonica* DSM 16522^T displayed very different molecular typing profiles (Pearson similarity coefficient of 10%), and for group G₈, in which strain TB20 displayed a unique molecular typing profile. Within group G₈, strains TB01, TB10 and TB30, all of which originated from China, clustered together and strains CA04 and USNY95, both isolated from *Steinernema kraussei*, and strain Si, isolated from *Steinernema intermedium*, were distinguished on the basis of their molecular typing profiles. In addition, the

DNA–DNA relatedness between *X. bovienii* T228^T (associated with *Steinernema feltiae*) and strain Si (associated with *S. intermedium*) was only 64 %, whereas the DNA relatedness between the *X. bovienii* type strain T228^T and strains F3 and SK2 (associated with *Steinernema affine* and *S. kraussei*, respectively) was 75 % (Boemare *et al.*, 1993). Within group G₉, *X. poinarii* strain CU01, isolated from *Steinernema cubanum*, was distinguished from the four *X. poinarii* strains isolated from *Steinernema glaseri*. Similarly, the DNA–DNA relatedness between *X. poinarii* G6^T (associated with *S. glaseri*) and strain CU01 (associated with *S. cubanum*) was only 68 %, with a ΔT_m value of 3 °C, whereas the DNA–DNA relatedness between *X. poinarii* G6^T and strain NC33 (associated with *S. glaseri*) was 96 %, with a ΔT_m value of 2.4 °C (Fischer-Le Saux *et al.*, 1999a). These results obtained using the DNA–DNA hybridization technique and our molecular typing method indicated the particular position of strains Si and CU01 within *X. bovienii* and *X. poinarii*, respectively. Strains TH01^T, ID10^T, VC01^T, PR06-A^T, USNJ01^T, Q1^T and *X. beddingii* DSM 4764^T, the 16S rRNA gene sequences of which were not included in any group, displayed unique molecular typing profiles.

Considering a Pearson similarity coefficient threshold above around 40 % to allow reliable assignment of a strain to a given group, the classification of our strains based on molecular typing profile analysis was consistent with that based on DNA–DNA hybridizations performed on representative strains of *X. beddingii*, *X. bovienii*, *X. japonica*, *X. nematophila* and *X. poinarii* and strains K77, SaV^T and Q1^T (Boemare *et al.*, 1993; Fischer-Le Saux *et al.*, 1999a; Nishimura *et al.*, 1994), thus indicating that our molecular typing technique has a level of resolution comparable to that of DNA–DNA hybridization. In addition, the groupings substantiate those obtained from analysis of 16S rRNA gene sequences. These results suggest that our approach is a reliable alternative to the quantitative DNA–DNA hybridization method generally used in bacterial taxonomy in cases in which a large number of bacterial strains with very similar 16S rRNA gene sequences are studied. Based on this approach, we were able (i) to classify 32 new isolates to the previously described *Xenorhabdus* species, adding new members to recently described species previously represented by the type strain only (Lengyel *et al.*, 2005; Somvanshi *et al.*, 2006), (ii) to confirm the validity of the five species proposed by Lengyel *et al.* (2005) and Somvanshi *et al.* (2006), despite the absence of quantitative DNA–DNA hybridization data and the high level of similarity of their 16S rRNA gene sequences (e.g. *X. budapestensis*–*X. ehlersii*, 98 %; *X. szentirmaii*–*X. nematophila*, 97.5 %), (iii) to propose the description of ten novel *Xenorhabdus* species (see below) and (iv) to assign 16 strains to these novel *Xenorhabdus* species, with isolates VN01 and TB20 remaining unclassified.

Comparison of phenotypic patterns

All the strains displayed the phenotypic characters of form I described previously for the genus *Xenorhabdus* (Akhurst & Boemare, 2005) with the exception of *X. ehlersii* DSM

16337^T and *X. japonica* DSM 16522^T, provided by the DSMZ, which contained form II only. All strains except *X. bovienii* BE05, *X. ehlersii* DSM 16337^T and *X. japonica* DSM 16522^T produced antibiotics and none of the strains displayed any of the following characters: bioluminescence, oxidase, catalase, urease or nitrate reductase activity, Voges–Proskauer reaction, ONPG (*o*-nitrophenol β -D-galactopyranoside) and H₂S production. Table 2 summarizes the most significant phenotypic features of the strains of the previously described *Xenorhabdus* species and of the ten novel *Xenorhabdus* species proposed here.

We compared the phenotypic patterns of the 76 strains studied here (Fig. 3). Similarity coefficients exceeded 70 %, suggesting that phenotypic analysis would be unlikely to lead to the identification of phenotypic features for reliable discrimination between *Xenorhabdus* species. A comparison of the phenotypic patterns led to the identification of two main phenotypic groups. Phenotypic group G_A comprised strains able to grow at temperatures of 35–42 °C, whereas phenotypic group G_B comprised strains growing at temperatures below 35 °C, suggesting that some *Xenorhabdus* species may have become adapted to tropical or temperate regions and/or influenced by the optimal growth and development temperature of their nematode host [e.g. the optimum temperatures for growth and development of *Steinernema carpocapsae*, *S. monticolum*, *S. scarabaei* and *S. weiseri*, associated with *X. nematophila*, *Xenorhabdus hominickii* sp. nov., *Xenorhabdus koppenhoeferi* sp. nov. and *X. bovienii* (group G_B), respectively, ranged from 20 to 25 °C, whereas those of *Steinernema riobrave* and *S. abbasi*, associated with *Xenorhabdus cabanillasii* sp. nov. and '*X. indica*' (group G_A), respectively, ranged from 30 to 35 °C (Cabanillas *et al.*, 1994; Elawad *et al.*, 1997; Mráček *et al.*, 2003; Saunders & Webster, 1999; Stock & Koppenhöfer, 2003; Stock *et al.*, 1997)]. The reliable phylogenetic relationships between strains of groups G₁ to G₄ (Fig. 1) were supported by the ability of the corresponding strains to grow at temperatures above 35 °C. Similarly, the phylogenetic relationships identified between strains ID10^T and the '*X. indica*' strains (group G₅) and between strain PR06-A^T and strains FRM16^T and FRG31 (*Xenorhabdus doucetiae* sp. nov., group G₁₁) were supported by the ability of these strains to grow at temperatures above 35 °C.

In the examples cited below, the classification of phenotypic patterns (Fig. 3) was generally consistent with the molecular classifications presented in Figs 1 and 2. The phenotypic patterns of the *X. nematophila* strains clustered together except for *X. nematophila* strain BE06, which was clustered with *X. japonica* DSM 16522^T (phase II). The *X. nematophila* strains were characterized by an ability to grow only at temperatures below 35 °C, to produce acid from trehalose and to assimilate fructose and an inability to assimilate D-gluconate. Colonies were not pigmented. The phenotypic patterns of the *X. bovienii* strains also clustered together except for strains CA04 and USNY95. Similarly, strain TB20 displayed a unique phenotypic pattern. The *X. bovienii*

Fig. 2. Comparison of the molecular typing profiles of the 76 *Xenorhabdus* strains and isolates studied. One ERIC profile and three RAPD profiles obtained with the primers P1, P2 and P3, used in independent reactions, were combined for each strain. The combined molecular typing profiles were compared using Pearson's similarity coefficient. The corresponding similarity matrix was used to generate a dendrogram using the UPGMA module of the GelCompar software (Applied Maths). Type strains are highlighted in bold. Molecular profiles were established for strains G6^T (synonym of DSM 4768^T), T228^T (synonym of DSM 4766^T), AN6^T (synonym of DSM 3370^T) and Q58^T (synonym of DSM 4764^T). When known, the *Steinernema* species of the corresponding nematode host is indicated. Groups G₁ to G₁₃ are indicated.

strains were characterized mainly by their ability to grow only at temperatures below 33 °C and their ability to assimilate D-gluconate (Table 2). Strain VN01 and *X. japonica* DSM 16522^T (group G₆) had very different phenotypic patterns (Fig. 3; Table 2) and therefore could not be considered to belong to the same species on the basis of our phenotypic and molecular analysis alone. Strains KE01^T, KR01 and KR05 (*X. hominickii*, group G₇) clustered together based on phenotypic patterns, confirming that

these strains belonged to the same species. The phenotypic patterns of the *X. poinarii* strains isolated from *S. glaseri* clustered together and were distinguished from the phenotypic pattern of *X. poinarii* CU01 isolated from *S. cubanum*. The *X. poinarii* strains were characterized mainly by their ability to grow at 40 °C, an absence of DNase and lecithinase activities and an inability to assimilate putrescine (Table 2). Strains JM26 and USTX62^T (*X. cabanillasii*, group G₁₃) clustered together on the basis of phenotypic

Table 2. Main phenotypic characters of the previously described *Xenorhabdus* species and of the ten proposed novel *Xenorhabdus* species determined in this study

Taxa: 1, *X. beddingii* Q58^T; 2, *X. bovienii* (24 strains studied); 3, unclassified strain TB20; 4, *X. budapestensis* (2 strains); 5, *X. ehlersii* (5 strains); 6, '*X. indica*' (2 strains); 7, *X. innexi* (2 strains); 8, *X. japonica* DSM 16522^T; 9, unclassified strain VN01; 10, *X. nematophila* (13 strains); 11, *X. poinarii* (5 strains); 12, *X. szentirmaii* (3 strains); 13, *X. cabanillasii* sp. nov. (2 strains); 14, *X. doucetiae* sp. nov. (2 strains); 15, *X. griffiniae* sp. nov. ID10^T; 16, *X. hominickii* sp. nov. (3 strains); 17, *X. koppenhoeferi* sp. nov. USNJ01^T; 18, *X. kozodoii* sp. nov. (3 strains); 19, *X. mauleonii* sp. nov. VC01^T; 20, *X. miraniensis* sp. nov. Q1^T; 21, *X. romanii* sp. nov. PR06-A^T; 22, *X. stockiae* sp. nov. TH01^T. +, 90 % of strains positive; v(+), 50–89 % of strains positive; v(–), 11–49 % of strains positive; –, 0–10 % of strains positive; v, variable; w, weak.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Maximum temperature for growth (°C)	39	32–33	33	37	38–40	39–41	40	34	37	34–35	40	37–39	39–40	40–42	39	33–35	33	38–41	40	38	37	39
Pigmentation	+	v(+)	+	–	v(+)	–	v	–	+	–	+	v(–)	v	+	–	+	+	+	+	+	+	+
DNase	+	+	+	+	v(–)	+	+	+	+	+	–	+	+	v	+	+	+	–	+	–	–	+
Simmons' citrate	+	v(–)	+	–	v(–)	–	v	–	–	v(–)	v(–)	v(+)	–	–	+	–	–	v(–)	+	+	–	–
Aesculin hydrolysis	+	–	–	v	v(+)	v	+	–	+	–	v(–)	+	–	v	+	+	–	v(–)	+	+	–	+
Lecithinase	+	v(+)	+	v	v(+)	v	+	–	+	v(+)	–	–	v	+	+	+	+	+	+	+	–	+
Acid production from:																						
Ribose	w	v(–)	–	–	v(+)	–	+	+	–	v(–)	–	v(–)	–	v	+	v(–)	–	v(+)	+	+	–	+
Inositol	–	v(–)	–	+	–	+	v	–	–	v(–)	–	v(+)	v	v	–	–	–	–	+	–	w	+
Sorbitol	–	–	–	–	–	–	–	–	–	–	–	v(+)	–	–	–	–	–	–	–	–	–	–
Maltose	+	v(+)	–	–	v(+)	–	–	+	+	v(+)	+	+	v	v	–	v(+)	+	+	+	+	+	+
Trehalose	+	v(–)	–	–	–	–	–	–	+	v(+)	v(+)	–	–	+	–	–	–	v(+)	+	+	+	–
Gluconate	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	+	w	w	+
5-Ketogluconate	+	v(–)	–	+	v(+)	+	v	+	+	v(–)	v(–)	–	v	–	w	+	+	–	w	w	w	w
Assimilation of:																						
Fructose	+	v(+)	+	+	v(–)	v	+	+	+	+	v(–)	–	v	v	–	v(–)	–	–	+	+	+	+
Maltotriose	+	+	+	v	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Maltose	+	v(+)	+	v	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Ribose	+	v(–)	+	–	v(–)	–	v	–	–	–	–	v(+)	–	v	–	v(–)	–	v(–)	+	+	–	+
Inositol	–	–	–	–	–	+	+	+	w	v(+)	–	+	–	+	w	–	–	–	+	–	+	+
D-Malate	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	+	–	–	+	+	w	w
cis-Aconitate	+	v(–)	+	–	v(+)	–	v	–	–	v(–)	v(+)	+	–	+	+	–	–	v(+)	+	+	+	–
Citrate	+	v(–)	+	–	v(+)	–	v	–	–	v(–)	+	+	+	+	+	–	–	+	+	+	+	–
D-Gluconate	+	+	+	v	v(+)	+	+	w	+	v(–)	+	+	–	+	+	+	–	v(–)	+	+	+	+
Putrescine	–	v(–)	–	–	v(+)	–	–	–	+	v(–)	–	v(+)	–	v	+	–	–	+	–	+	w	–
Lactate	+	v(+)	+	+	v(+)	+	+	w	+	+	+	+	–	+	+	v(+)	–	+	+	+	+	+
Histidine	+	v(–)	–	–	v(–)	–	v	–	–	–	v(–)	–	v	v	–	–	–	v(–)	–	–	–	–
Succinate	+	v(–)	w	–	v(+)	+	+	+	w	v(+)	v(+)	+	–	+	+	v(+)	–	+	+	+	+	+
D-Glucosamine	+	v(–)	+	–	v(–)	v	v	–	–	v(–)	v(–)	v(+)	–	v	–	–	–	v(–)	–	+	–	+
L-Aspartate	+	v(+)	+	–	+	+	+	+	w	+	v(+)	+	v	+	+	+	–	v(+)	+	+	+	+
D-Alanine	+	v(+)	+	–	v(–)	+	+	+	–	v(+)	v(+)	+	–	+	+	w	–	v(+)	+	+	+	+
Propionate	+	–	+	–	v(–)	–	–	w	–	v(–)	v(–)	v(–)	–	–	–	–	–	–	w	w	w	–
Tyrosine	+	v(–)	–	–	v(–)	v	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+

patterns, confirming that they belonged to the same species. Thus, at least for these strains, phenotypic analysis confirmed the high reliability of our molecular classification of the *Xenorhabdus* strains.

For groups G₄ and G₁₂, the phenotypic patterns of the corresponding strains were not grouped together in hierarchical analysis (Fig. 3). However, for group G₄ (*Xenorhabdus kozodoii* sp. nov.), composed of three strains

Fig. 3. Comparison of the phenotypic patterns of the 76 *Xenorhabdus* strains and isolates studied. Five similarity matrices were calculated on the basis of variable characters only, each corresponding to a set of data: growth at different temperatures, enzyme activities, carbon source assimilation and fermentation and phenotypic tests on agar plates. The phenotypic patterns were compared using the Gower similarity coefficient, making it possible to include weakly positive signals, recorded as 0.5. The five similarity matrices were combined using BioNumerics software (Applied Maths) and the resulting dendrogram was calculated using UPGMA. Type strains are highlighted in bold. Phenotypic patterns were established for strains G6^T (synonym of DSM 4768^T), T228^T (synonym of DSM 4766^T), AN6^T (synonym of DSM 3370^T) and Q58^T (synonym of DSM 4764^T). When known, the *Steinernema* species of the corresponding nematode host is indicated. Groups G₁ to G₁₃ and phenotypic groups G_A and G_B are indicated.

originating from different countries (Italy, Russia, Spain), a combination of phenotypic features (proposed in the species description below) was found to distinguish these strains from the other *Xenorhabdus* strains studied (Table 2). Similarly, for group G₁₂, composed of three strains, including the type strain *X. szentirmaii* DSM 16338^T, a combination of phenotypic features was used to distinguish these three strains unambiguously from the other *Xenorhabdus* strains studied [acid produced from sorbitol, assimilation of D(+)-malate, no lecithinase activity and no assimilation of fructose]. Unlike strain DSM 16348^T, strains AR81 and K77 were able to assimilate ribose and to produce acid from fructose, mannose and inositol.

For each of groups G₁ (*X. ehlersii*), G₂ (*X. budapestensis*), G₃ (*X. innexi*), G₅ (*X. indica*) and G₁₁ (*X. doucetiae*), the phenotypic patterns of the corresponding strains were not grouped together (Fig. 3) and no phenotypic feature could be identified that distinguished these groups unambiguously from the other *Xenorhabdus* strains studied. However, the biochemical characteristics of the type strain *X. ehlersii* DSM 16337^T defined by Lengyel *et al.* (2005) were shared by the four new isolates that we assigned to this species, with the exception of assimilation of fructose, arabinose, xylose, rhamnose, phenylacetate and tyrosine, displayed by none of the four isolates. Unlike the type strain *X. budapestensis* DSM 16342^T (Lengyel *et al.*, 2005), strain CN03 produced indole, hydrolysed aesculin, produced acid from acetylglucosamine and assimilated maltotriose, maltose, *N*-acetyl-D-glucosamine, D-gluconate, L-glutamate, L-proline, L-alanine and L-serine. The biochemical characteristics of the type strain *X. innexi* DSM 16336^T (Lengyel *et al.*, 2005) were shared by strain UY61, but UY61 was also able to assimilate Simmons' citrate and histidine, to produce indole and to ferment inositol. The biochemical characteristics of the proposed type strain '*X. indica*' DSM 17382 (Somvanshi *et al.*, 2006) were shared by strain OM01, but OM01 was also able to assimilate *N*-acetyl-D-glucosamine and tyrosine and to hydrolyse aesculin. No combination of phenotypic traits was identified that distinguished strains TH01^T, ID10^T, USNJ01^T, VC01^T, Q1^T, PR06-A^T, *X. japonica* DSM 16522^T or *X. beddingii* DSM 4764^T from the other *Xenorhabdus* species (Table 2).

Description of *Xenorhabdus cabanillasii* sp. nov.

Xenorhabdus cabanillasii [ca.ba.nil'la.si.i. N.L. gen. masc. n. *cabanillasii* in honour of H. E. Cabanillas, who described the

nematode host of this bacterium, *Steinernema riobrave* (Cabanillas *et al.*, 1994)].

The upper temperature limiting growth of the two known strains of this species in LB broth lies between 39 and 40 °C. Assimilates Simmons' citrate, does not hydrolyse aesculin, does not ferment ribose, sorbitol, trehalose or gluconate and does not assimilate ribose, inositol, D-malate, *cis*-aconitate, D-gluconate, putrescine, lactate, succinate, D-glucosamine, propionate or tyrosine. Known strains are symbiotically associated with the entomopathogenic nematode *Steinernema riobrave*, from which two geographical ecotypes have been isolated, USTX62^T from Texas (USA) and JM26 from the Caribbean island of Jamaica.

The type strain is strain USTX62^T (= CIP 109066^T = DSM 17905^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is AY521244.

Description of *Xenorhabdus doucetiae* sp. nov.

Xenorhabdus doucetiae [dou.ce'ti.ae. N.L. gen. fem. n. *doucetiae* in honour of M. M. A. Doucet, who described the first *Steinernema* species from South America (Doucet, 1986)].

The upper temperature limiting growth of the two known strains of this species in LB broth lies between 40 and 42 °C. Produces acid from trehalose and assimilates inositol, *cis*-aconitate and citrate. Known strains are symbiotically associated with the entomopathogenic nematode *Steinernema diaprepesi* (Nguyen & Duncan, 2002), isolated in Central America and the Caribbean islands.

The type strain is strain FRM16^T (= CIP 109074^T = DSM 17909^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211709.

Description of *Xenorhabdus griffiniae* sp. nov.

Xenorhabdus griffiniae [grif.fi'ni.ae. N.L. gen. fem. n. *griffiniae* in honour of C. T. Griffin, who has contributed to the systematics of the nematode host, *Steinernema hermaphroditum* (Stock *et al.*, 2004)].

The upper threshold limiting growth in LB broth is 39 °C. Colonies are not pigmented. Does not acidify inositol, sorbitol, maltose, trehalose or gluconate. Assimilates putrescine. The type strain is symbiotically associated with

the entomopathogenic nematode *Steinernema hermaphroditum*, isolated in Indonesia.

The type strain is strain ID10^T (=CIP 109073^T=DSM 17911^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211710.

Description of *Xenorhabdus hominickii* sp. nov.

Xenorhabdus hominickii (ho.mi.ni'cki.i. N.L. gen. masc. n. *hominickii* in honour of W. M. Hominick, who has contributed to the systematics of entomopathogenic nematode–bacterium complexes).

The maximum temperature at which the three known strains of this species can grow is between 33 and 35 °C. Colonies are yellow. Produces indole, an exceptional characteristic in the genus *Xenorhabdus*, and hydrolyses aesculin. Produces acid from D-malate and 5-ketogluconate and does not assimilate Simmons' citrate, citrate, *cis*-aconitate, inositol or D-glucosamine. The isolates of this species are symbiotically associated with the entomopathogenic nematodes *Steinernema kari* (Waturu *et al.*, 1997) and *Steinernema monticolum* (Stock *et al.*, 1997), from Kenya and Korea, respectively.

The type strain is strain KE01^T (=CIP 109072^T=DSM 17903^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211719.

Description of *Xenorhabdus koppenhoeferi* sp. nov.

Xenorhabdus koppenhoeferi [kop.pen.hoe'fe.ri. N.L. gen. masc. n. *koppenhoeferi* in honour of A. Koppenhöfer, who has contributed to the systematics of the nematode host, *Steinernema scarabaei* (Stock & Koppenhöfer, 2003)].

The upper temperature limiting growth in LB broth is 33 °C. Colonies are yellow. Produces acid from maltose and 5-ketogluconate. The type strain is symbiotically associated with the entomopathogenic nematode *Steinernema scarabaei*, isolated from New Jersey (USA).

The type strain is strain USNJ01^T (=CIP 109199^T=DSM 18168^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ205450.

Description of *Xenorhabdus kozodoii* sp. nov.

Xenorhabdus kozodoii [ko.zo'do.i.i. N.L. gen. masc. n. *kozodoii* in honour of E. M. Kozodoi, who described the nematode host *Steinernema arenarium* (*Neoplectana anomali*) (Kozodoi, 1984)].

The upper temperature limiting growth of the three known strains of this species in LB broth is between 38 and 41 °C. Produces acid from gluconate and assimilates putrescine. Has no DNase activity and does not assimilate fructose. Does not produce acid from 5-ketogluconate. Known strains are symbiotically associated with the entomopathogenic nematodes *Steinernema arenarium* and *Steinernema*

apuliae (Triggiani *et al.*, 2004), isolated in Russia and Italy, respectively.

The type strain is strain SaV^T (=CIP 109068^T=DSM 17907^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211716.

Description of *Xenorhabdus mauleonii* sp. nov.

Xenorhabdus mauleonii (mau.le.o'ni.i. N.L. gen. masc. n. *mauleonii* in honour of H. Mauléon, who has made a major contribution to studies of the ecology and biodiversity of entomopathogenic nematode–bacterium complexes in the Caribbean region).

The upper temperature limiting growth in LB broth is 40 °C. Colonies are yellow and assimilate Simmons' citrate and D-malate. Does not assimilate D-glucosamine or tyrosine. The type strain is symbiotically associated with an as-yet-unidentified species of *Steinernema* isolated from the Caribbean island of St. Vincent.

The type strain is strain VC01^T (=CIP 109075^T=DSM 17908^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211715.

Description of *Xenorhabdus miraniensis* sp. nov.

Xenorhabdus miraniensis (mi.ra.ni.en'sis. N.L. fem. adj. *miraniensis* from Mirani, a small town in Australia, the source of the nematode from which the type strain was isolated).

The upper temperature limiting growth in LB broth is 38 °C. Has no DNase activity and assimilates Simmons' citrate and many carbohydrates, including D-malate, lactate, D-glucosamine, L-aspartate and L-tyrosine. The type strain is symbiotically associated with an as-yet-undescribed nematode of the Steinernematidae (Akhurst & Boemare, 1988) isolated at Mirani in Queensland (Australia) and has not yet been found elsewhere.

The type strain is strain Q1^T (=CIP 109069^T=DSM 17902^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211713.

Description of *Xenorhabdus romanii* sp. nov.

Xenorhabdus romanii [ro.ma'ni.i. N.L. gen. masc. n. *romanii* in honour of J. Román, who described the nematode host *Steinernema puertoricense* (Román & Figueroa, 1994)].

The upper temperature limiting growth in LB broth is 37 °C. Colonies are yellow and display no DNase activity or aesculin hydrolysis. Does not assimilate histidine and assimilates D-malate inefficiently. The type strain is symbiotically associated with the entomopathogenic nematode *Steinernema puertoricense*, isolated in Puerto Rico.

The type strain is strain PR06-A^T (= CIP 109070^T = DSM 17910^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ211717.

Description of *Xenorhabdus stockiae* sp. nov.

Xenorhabdus stockiae [sto'cki.æ. N.L. gen. fem. n. *stockiae* in honour of S. P. Stock, a leading figure in the systematics of *Steinernema* and particularly of the nematode host of this bacterium, *Steinernema siamkayai* (Stock *et al.*, 1998)].

The upper temperature limiting growth in LB broth is 39 °C. Colonies are pink. Assimilates D-glucosamine and tyrosine in particular, but does not produce acid from trehalose. The type strain is symbiotically associated with the entomopathogenic nematode *Steinernema siamkayai*, isolated in Thailand.

The type strain is strain TH01^T (= CIP 109067^T = DSM 17904^T). The GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ202309.

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