Phylogenetic Evidence for the Taxonomic Heterogeneity of *Photorhabdus luminescens*

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The sequences of the 16S rRNA gene of 40 strains of bacterial symbionts isolated from the nematodes *Heterorhabditis* spp. and seven bacterial symbionts of the nematodes *Steinernema* spp. which were isolated from different geographical areas, as well as the type strain of *Xenorhabdus japonicus*, were determined and compared to each other and to the sequences of several reference strains of members of the *Enterobacteriaceae*. The data confirmed the separate status of the two genera of symbionts of entomopathogenic rhabditid nematodes. The symbionts of *Heterorhabditis* spp. clustered with the type strain of *Photorhabdus luminescens*, while the symbionts of *Steinernema* spp. grouped with *Xenorhabdus* species. *X. japonicus* clustered with the other *Xenorhabdus* species. Phylogenetic analysis of 15 almost complete 16S ribosomal DNA (rDNA) sequences of the *Heterorhabditis* symbionts indicated that there were several subclusters. The properties correlated with these subclusters are not yet apparent, although there may be some geographical and ecological correlations. For example, among the nematode-symbiotic bacteria, the members of subclusters I and III are from southeastern and midwestern North America, respectively, while the members of subclusters II and IV are primarily from Europe and Australia, respectively. The nonsymbiotic strains of *P. luminescens* form a highly homologous subcluster by themselves. The results of DNA-DNA hybridization studies performed with a few selected strains of five of the 16S rDNA subclusters support the existence of several genospecies within *P. luminescens*.

Entomopathogenic bacteria that are symbiotic with nematodes in the genera *Heterorhabditis* and *Steinernema* have been known for many years (1, 3, 15, 26), and originally these insect-pathogenic bacteria were placed in the genus *Xenorhabdus*, with the luminous forms affiliated with the species *Xenorhabdus luminescens* and the nonluminous forms affiliated with several different species (*Xenorhabdus nematophilus*, *Xenorhabdus beddingii*, *Xenorhabdus bovienii*, *Xenorhabdus japonicus*, and *Xenorhabdus poinarii*). It was later proposed on the basis of physiological and genomic (DNA-DNA hybridization) analysis data that *X. luminescens* should be given genus status, and this taxon was designated *Photorhabdus luminescens* (6). All symbiotic *P. luminescens* strains to date have been isolated from nematodes belonging to the heterorhabditis group, while the *Xenorhabdus* species have been obtained from nematodes belonging to the genus *Steinernema*. Recently, several nonsymbiotic isolates of *X. luminescens* were described (13). These strains were obtained from human wounds, and while they share many properties with other *X. luminescens* strains, they are apparently not associated with nematodes. Although the genus *Photorhabdus* has been proposed (6), many of the details concerning the phylogeny and taxonomy of the genera *Xenorhabdus* and *Photorhabdus* have not yet been settled (23). The issues of phylogeny, taxonomy, and identification of strains take on importance for the following reasons: (i) these bacteria are economically important because of their ability to kill a variety of insect prey (9–11); (ii) these bacteria are often (but not always) symbiotically associated with nematode hosts, and the nature of the host-bacterium symbiosis has not yet been

precisely defined; (iii) these bacteria form phase variants (2, 4, 5) that are difficult to identify but economically important because of the formation of symbiotic relationships with the infective forms of their nematode hosts and the ability of the bacteria to support efficient nematode growth; and (iv) these bacteria have also been isolated (as free-living forms) from human wounds (13), suggesting that their ecology, as a group, may be more complex than previously imagined. With regard to the last point, no analyses of the 16S ribosomal DNA (rDNA) genes of the human wound isolates have been described yet.

In previous work on these groups of bacteria, Rainey et al. (23) were unable to unambiguously separate the genera *Xenorhabdus* and *Photorhabdus* on the basis of 16S rDNA sequence analyses and suggested that the addition of more strains, especially strains of *P. luminescens*, might allow workers to settle the issue of genus status. Accordingly, here we present data from 16S rDNA analyses of several other *P. luminescens* strains, including isolates from Europe, North America, Australia, and Israel, as well as both symbiotic and nonsymbiotic isolates. The addition of these strains resulted in a data set that allows us to support with more certainty the separation of these organisms into two genera, and furthermore, the data suggest that there may be some subclusters worthy of genospecies rank within the species *P. luminescens*.

MATERIALS AND METHODS

Bacterial strains. 16S rDNAs were isolated from 40 *Photorhabdus* strains, *X. japonicus* IAM 14265^T (T = type strain) (21), three strains of *X. nematophilus*, and four strains of *X. bovienii*. The designations, origins, and hosts of the strains, the lengths of the 16S rDNA stretches analyzed and the 16S rDNA accession numbers are listed in Table 1. The sequences of *P. luminescens* DSM 3368^T (accession number X82248), DSM 3369 (X82249), and HSH2 (X82250), *X. nematophilus* DSM 3370^T (X82251), *X. bovienii* DSM 4766^T (X82252), *X. poinarii* DSM 4768^T (X82253), *X. beddingii* DSM 4764^T (X82254), *Citrobacter freundii* (M59291), *Erwinia carotovora* (M59149), *Escherichia coli* (M24911), *Hafnia alvei*

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TABLE 1. Designations, origins, eukaryotic hosts, and 16S rDNA accession numbers of the strains analyzed in this study and the lengths of the 16S rDNA sequences determined

Strain	Source	Origin of host	Host	Sequence length (nucleotides)	Accession no.
<i>Photorhabdus</i> strains					
WX1	K. H. Nealson	Wisconsin	Unknown	469	Z77195
WX1F	K. H. Nealson	Wisconsin	Unknown	469	Z77196
WX2	K. H. Nealson	Wisconsin	Unknown	1,439	Z76746
WX3	K. H. Nealson	Wisconsin	Unknown	469	Z77197
WX4	K. H. Nealson	Wisconsin	Unknown	469	Z77198
WX5	K. H. Nealson	Wisconsin	Unknown	469	Z77199
WX6	K. H. Nealson	Wisconsin	Unknown	469	Z77200
WX8	K. H. Nealson	Wisconsin	Unknown	469	Z77201
WX9	K. H. Nealson	Wisconsin	Unknown	469	Z77202
WX10	K. H. Nealson	Wisconsin	Unknown	469	Z77203
WX11	K. H. Nealson	Wisconsin	Unknown	1,439	Z76747
WX12	K. H. Nealson	Wisconsin	Unknown	469	Z77204
WX13Z	K. H. Nealson	Wisconsin	Unknown	469	Z77205
WX14	K. H. Nealson	Wisconsin	Unknown	469	Z77206
WX15	K. H. Nealson	Wisconsin	Unknown	469	Z77207
V16	K. H. Nealson	Australia	<i>Heterorhabditis bacteriophora</i>	1,444	Z76741
Q614	K. H. Nealson	Australia	Unknown	1,452	Z76749
Tn-5*2	K. H. Nealson	Florida	Unknown	1,428	Z76740
Hm, 1° and 2°	K. H. Nealson ^a	Georgia	<i>H. bacteriophora</i>	1,439	Z76742
Hm Hyper ^b	K. H. Nealson	Georgia	<i>H. bacteriophora</i>	469	Z77208
HP88	R. Georgis	Utah	<i>H. bacteriophora</i>	1,439	Z76743
IS-5 ^c	I. Glazer	Israel	<i>H. bacteriophora</i>	1,439	Z76745
Moldavian	L. Gerritsen	Moldavia	<i>H. bacteriophora</i>	469	Z77186
Brecon ^c	L. Gerritsen	South Australia	<i>H. bacteriophora</i>	1,439	Z76744
Meg1	K. H. Nealson	Ohio	<i>Heterorhabditis megidis</i>	1,440	Z76750
Meg2	K. H. Nealson	Ohio	<i>H. megidis</i>	1,446	Z76751
HSH1, 1° and 2°	K. Osterfeld	Germany	<i>H. megidis</i>	469	Z77188
HSH3, 1° and 2°	K. Osterfeld	Germany	<i>H. megidis</i>	469	Z77189
UK 211 ^c	A. Burnell	Great Britain	<i>H. megidis</i>	469	Z77193
HL81 ^c	P. Westerman	The Netherlands	<i>H. megidis</i>	469	Z77190
PE 87.3 ^c	P. Gerritsen	The Netherlands	<i>H. megidis</i>	1,439	Z76748
PE 87.3, var PE pink	L. Gerritsen	The Netherlands	<i>H. megidis</i>	469	Z77191
PE87.3, var PE yellow	L. Gerritsen	The Netherlands	<i>H. megidis</i>	469	Z77192
PEGB	L. Gerritsen	The Netherlands	<i>H. megidis</i>	469	Z77187
CHJG 1	J. Grund	Switzerland	<i>H. megidis</i>	469	Z77194
P-Jun	L. Gerritsen	The Netherlands	<i>H. megidis</i>	467	Z77185
ATCC 43949	K. H. Nealson	CDC ^d	Human blood	1,428	Z76752
ATCC 43950	K. H. Nealson	CDC	Human pretibial wound	1,428	Z76755
ATCC 43951	K. H. Nealson	CDC	Leg wound	1,426	Z76754
ATCC 43952	K. H. Nealson	CDC	Abdomen, submandible	1,426	Z76753
<i>Xenorhabdus nematophilus</i> strains					
703	K. Nealson		<i>Steinernema carpocapsae</i>	467	Z77209
N2-4, 1° and 2°	R. Georgis	Biosys	<i>S. carpocapsae</i> strain Mexicana	1,437	Z76737
Riobravis	P. Grewal	New Mexico	<i>Steinernema riobrava</i>	1,437	Z76738
<i>Xenorhabdus bovienii</i> strains					
SF22 ^c	L. Gerritsen	Finland	<i>Steinernema feltiae</i>	467	Z77210
Norv ^c	L. Gerritsen	Norway	<i>S. feltiae</i>	467	Z77211
Sulc ^c	L. Gerritsen	Unknown	<i>S. feltiae</i>	467	Z77212
Filip ^c	L. Gerritsen	Unknown	<i>S. feltiae</i>	467	Z77213
<i>Xenorhabdus japonicus</i>	Japanese Culture Collection	Japan	<i>Steinernema kushidai</i>	1,389	Z76739
IAM 14265 ^T					

^a According to reference 4.^b Strain Hm Hyper is a mutant of the strain Hm 2° form which has all of the 1° form characteristics.^c Strain isolated by E. Szállás.^d CDC, Centers for Disease Control and Prevention, Atlanta, Ga.

(M59155), *Pantoea agglomerans* (L24784), *Serratia marcescens* (M59160), *Yersinia enterocolitica* (M59292), and *Proteus vulgaris* (X07652) were used as references. The reference sequences were obtained from the Ribosomal Database Project (20).

Analysis of 16S rDNA. Amplification of the 16S rDNA was carried out as described previously (22). PCR products were sequenced directly by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) according to the manufacturer's protocol. The sequence reaction mixtures

were electrophoresed by using an Applied Biosystems model 373A DNA sequencer.

Phylogenetic analysis. The 16S rDNA sequences were compared with the existing 16S rDNA database for members of the family *Enterobacteriaceae* deposited in the Ribosomal Database Project (20). Similarity values were transformed into phylogenetic distance values that compensated for multiple substitutions at any given site in the sequence (19). The least-squares distance method of De Soete (8) and the Neighbor Joining (24) and Maximum Likelihood pro-

grams contained in the PHYLIP package (14) were used in the construction of phylogenetic dendrograms. For calculation of bootstrap values 300 trees were analyzed by using the NJFIND and NJBOOT programs.

DNA-DNA reassociation studies. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (7). DNA-DNA hybridization was carried out as described by Huß et al. (17) and Escara and Hutton (12) by using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (18).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the EMBL database under accession numbers Z76741 to Z76755 (for almost complete sequences) and Z77195 to Z77213 (for partial sequences) (Table 1).

RESULTS

Analysis of almost complete 16S rDNA sequences. Almost complete 16S rDNA sequences ranging in length from 1,428 to 1,452 nucleotides (93 to 96% of the *E. coli* 16S rDNA sequence) were determined for 16 luminescent strains and two nonluminescent strains. The sequence of a stretch of 1,339 nucleotides of the 16S rDNA of *X. japonicus* IAM 14265^T was also determined. These sequences were compared to homologous sequences from the type strains of *P. luminescens*, *X. nematophilus*, *X. bovienii*, *X. beddingii*, and *X. poinarii*, two strains of *P. luminescens* (DSM 3369 and HSH2) (23), and one strain each of several species of the family Enterobacteriaceae. No differences were detected in the primary structures of the 16S rDNAs of the primary phase variant (1°) and secondary phase variant (2°) of strain Hm and *X. nematophilus* N2-4.

The similarity values determined for the luminescent strains indicate that there is a high level of relatedness between the type strain of *P. luminescens* and the two previously analyzed strains of this species (levels of similarity, >96%). The two nonluminescent strains, strain Riobravus and the 1° form of strain N2-4, are highly related to the type strain of *X. nematophilus* (levels of similarity, >99.5%). *X. japonicus* type strain IAM 14265 and the type strains of the other four *Xenorhabdus* species are separated by similarity values which range from 96.4 to 97.8%. All *Xenorhabdus* strains could be clearly distinguished from strains of *P. luminescens* by the occurrence of a signature nucleotide sequence between positions 208 and 211 (*E. coli* numbering) of the 16S rDNA. While strains of *Xenorhabdus* species have the sequence UUCG between these positions, all luminescent strains have a longer version (UGAAAG). The levels of 16S rDNA sequence similarity between the two genera ranged from 94 to 96%, while the values determined for levels of similarity between strains of the genera *Xenorhabdus* and *Photobacterium* and strains of other members of the enterobacteria were between 92 and 95%. When the sequence of *Proteus vulgaris* was used as a root, phylogenetic trees based on distance matrix analysis data and maximum-likelihood analysis data grouped the genera *Xenorhabdus* and *Photobacterium* as phylogenetic neighbors within the family Enterobacteriaceae. The two algorithms used produced similar patterns of relatedness, except for the branch point of *X. beddingii*. According to the distance matrix tree, all *Xenorhabdus* species formed a phylogenetically coherent cluster, while in the neighbor-joining tree *X. beddingii* formed an individual line of descent between *X. nematophilus* and the *Photobacterium* cluster. In the distance matrix tree (Fig. 1), the bootstrap value of 63% obtained for the branching pattern of the *Xenorhabdus* cluster was significantly higher than the value obtained for the branching point of *Xenorhabdus* species (8%), as determined by the neighbor-joining method (data not shown). Therefore, the relationships of the symbionts of entomopathogenic rhabditid nematodes are depicted in a distance matrix tree (Fig. 1).

All luminescent strains grouped with the type strain of *P.*

FIG. 1. Phylogenetic tree showing the relationships among members of the genera *Xenorhabdus* and *Photobacterium* and members of the family Enterobacteriaceae. The analysis was based on 1,436 nucleotides of the 16S rDNA. Strains separated by a comma have identical sequences. The sequence of *Proteus vulgaris* served as the root. Bar = 2 nucleotide substitutions per 100 nucleotides. The numbers are bootstrap values.

luminescens, and this cluster was clearly separated from the *Xenorhabdus* species (bootstrap value, 100%). The isolates of *P. luminescens* formed six related subclusters (subclusters I to VI), most of which were supported by bootstrap values of more than 98%. Subcluster I contained the type strain of the species (DSM 3368), as well as strains Hm and strain Tn-5*2, which were isolated in Georgia and Florida, respectively (level of similarity, 99.8%). Subcluster II contained strain HSH2 (from Germany), which was analyzed previously (23), and strain PE-87.3 (from The Netherlands) (level of similarity, 99.5%). The phylogenetic neighbor subcluster III contained previously analyzed strain DSM 3369 (origin unknown), strains WX2 and WX11 (isolated in Wisconsin), and strains Meg1 and Meg2, which are independent isolates from the same location in Ohio (level of similarity, more than 98.8%). Subcluster IV was composed of strains V16, Q614, and Brecon (all from Australia) and strain HP-88 (from Utah) (level of 98.9% similarity, more than 98.9%). Strain IS5 (from Israel) formed subcluster V, which had a low bootstrap value for the branch point of members of subclass IV (53%). The 16S rDNA sequences of the four American Type Culture Collection strains from human

FIG. 2. Phylogenetic tree showing the relationships among members of the genera *Xenorhabdus* and *Photorhabdus*. The analysis was based on 469 nucleotides of the 16S rDNA. Strains separated by commas have identical sequences. The sequence of *Proteus vulgaris* served as the root. Bar = five nucleotide substitutions per 100 nucleotides.

wounds and lesions were identical, and because these strains clustered separately from all other *P. luminescens* subclusters, they were described as members of subcluster VI.

Analyses of partial 16S rDNA sequences. In order to extend the phylogenetic analyses of *P. luminescens* strains, about 460 nucleotides from the 5' terminus of the 16S rDNA were analyzed for an additional 24 strains that were originally isolated from diverse areas (Great Britain, central Europe, Israel, North America [Wisconsin, Ohio, Florida, and Texas], and Australia); all of these strains were isolated from nematodes. In order to determine the phylogenetic depth of the species of the genus *Xenorhabdus*, isolates of *X. nematophilus* and *X. bovienii* were also included in this study. No differences were detected in the primary structures of the 16S rDNAs of the 1^o and 2^o forms of strains HSH1 and HSH3, and there were no differences between the color variants of strain PE87.3. By using the sequences of two *Xenorhabdus* species to root the *Photorhabdus* cluster, the phylogenetic coherence of the luminescent strains was demonstrated with both of the algorithms used. A distance matrix tree is shown Fig. 2. All of the subclusters defined by the analysis of almost complete sequences were recovered, although the order of subclusters and the intersubcluster relatedness of strains changed. The branch point of subclusters I and II was supported by moderately high bootstrap values (<60 to 80%). Several strains of subcluster II, which contained the majority of the symbionts of the north-western European strains of *Heterorhabditis megidis*, were

found to have identical 16S rDNA sequences in the stretch analyzed; these strains included strains from The Netherlands (P87.3), Switzerland (CHJG1), the United Kingdom (UK211) and Germany (HSH3). Subcluster III was composed mainly of Wisconsin (WX) strains, whose 16S rDNAs appear to be phylogenetically slightly more heterogeneous (level of dissimilarity, 2.5%) than the 16S rDNAs of the European strains (level of dissimilarity, 1%). It should be noted that the Wisconsin strains came from different sites, and even from different soil habitats (prairie, birch trees, elm trees) and that the strains were isolated in separate baiting experiments on different days. The sites ranged over an area about 300 km in diameter, and the closest two sites were about 40 km from each other.

In addition to the subclusters identified on the basis of almost complete and partial 16S rDNA sequences, two new lineages emerged when additional strains were included. European strain Moldavian, isolated from *Heterorhabditis bacteriophora*, had a unique phylogenetic position. Strain P-Jun, isolated from The Netherlands, is an interesting organism as its branch point was between members of the genera *Photorhabdus* and *Xenorhabdus*. Based on biochemical evidence, this strain is an authentic member of the genus *Photorhabdus*, but its 16S rDNA has the signature sequence UUCG (positions 208 to 211) which is found in the 16S rDNA sequences of *Xenorhabdus* species.

DNA-DNA reassociation experiments. In order to determine whether the subclusters that emerged from the analysis of a single gene could be recovered when genomic similarities were determined, DNA-DNA reassociation experiments were performed with selected strains from rDNA subclusters I to V (Table 2). These experiments were necessary as (i) DNA-DNA similarity values are needed to delimit species in the polyphasic approach to classification (27) and (ii) DNA similarities cannot be extrapolated from 16S rDNA similarities because of the non-linear correlation between these two taxonomic parameters (25). The type strain of *P. luminescens* strain DSM 3368, and its relatives (subcluster I) exhibited moderately high levels of reassociation with members of subcluster IV (levels of similarity, 56 to 74%). Similar values were obtained for members of subclusters II and III (levels of similarity, 56 to 76%). Strain IS5, a member of subcluster V, exhibited moderately high levels of reassociation with members of subclusters I, III, and IV (levels of similarity, 59 to 71%). All other intersubcluster values were lower, ranging from 40 to 66% DNA similarity. The DNA similarity values determined for selected strains of *P. luminescens* and *E. coli* were less than 20% (data not shown).

DISCUSSION

Previous 16S rDNA analysis of four type strains of *Xenorhabdus* species and three strains of *P. luminescens* did not clearly separate the monospecific genus *Photorhabdus* from members of the genus *Xenorhabdus* (23). The branch point of *P. luminescens* and the branch points of the *Xenorhabdus* strains changed with the treeing algorithm used, and no conclusion could be drawn concerning whether the genus *Xenorhabdus* is a monophyletic taxon. Rainey et al. (23) discussed the possibility that the data set of available 16S rDNA sequences for *Xenorhabdus* and *Photorhabdus* strains was too small to allow the algorithms to generate stable branching patterns for these highly related strains. Furthermore, the phylogenetic diversity of a species (e.g., the range of levels of 16S rDNA dissimilarity among its strains) and the phylogenetic coherence of a species cannot be assessed if only the type strain of a species is included in 16S rDNA studies. Analysis of the

TABLE 2. Levels of DNA-DNA reassociation between representative strains of five *P. luminescens* 16S rDNA subclusters

Subcluster	Strain	% DNA-DNA reassociation with:										
		Hm, 1°	Hm, 2°	Hm Hyper	UK211	CHJG I	WX8	WX12	WX13Z	HP88	Brecon	IS-5 ^a
I	DSM 3368 ^T	100	ND ^b	100	45	46	49	52	47	60	56	70
	Hm, 1°		ND	95	53	46	50	63	63	74	ND	67
	Hm, 2°			99	ND	ND	46	59	ND	ND	ND	ND
	Hm Hyper				ND	ND	ND	ND	ND	ND	ND	ND
II	UK211					93	65	62	60	47	ND	61
	CHJG I						74	76	56	52	ND	59
III	WX8B							88	88	46	ND	70
	WX12								83	55	ND	63
	WX13Z									55	ND	66
IV	HP88										77	71
	Brecon											ND

^a Strain IS-5 is a subcluster V strain.^b ND, not determined.

significantly enlarged data set generated in this study clearly indicated the phylogenetic separateness of the two genera.

The clustering of strains by and large correlates with the origins of the host nematodes. Among the nematode-symbiotic bacteria, subcluster I and III organisms are from southeastern and midwestern North America, respectively, while subcluster II and IV organisms are primarily from Europe and Australia, respectively. On the other hand, subcluster V is composed of strains from Israel and Wisconsin. The phylogenetic stability of two new sublines identified by partial 16S rDNA analysis (strains Moldavian and P-Jun) needs to be tested by analyzing complete 16S rDNA sequences. Strain P-Jun, isolated in The Netherlands from a *H. megidis* host, represents the most deeply branching strain of all luminous strains investigated in this study. The presence of the enterobacterium-*Xenorhabdus* 16S rDNA signature sequence at positions 208 to 211 together with the ability to exhibit fluorescence describes a new combination not previously detected. Strain P-Jun may represent a new species once more relatives have been isolated and studied by a polyphasic approach.

The four strains of subcluster VI are the only *Photorhabdus* strains for which a symbiotic association with entomopathogenic nematodes has not been demonstrated. These strains have identical 16S rDNA sequences, although the strains were isolated from different humans. Whether the patients were originally infected by nematode-bacterium-contaminated material or whether the *Photorhabdus* strains are free-living, opportunistic representatives of the genus cannot be determined yet.

The range of dissimilarity values for strains of subclusters I to V was as large as the range found for strains of *X. nematophilus* and *X. bovienii*. In order to decide whether these subclusters may correlate with genospecies, DNA reassociation studies were performed with a small number of representatives of each subcluster. Most binary intrasubcluster DNA similarity values were greater than 70%, while most intersubcluster DNA similarity values were less than or around 70%. The genomic heterogeneity of *Photorhabdus* strains confirmed previous work that demonstrated that there are individual subgroups within this species. Grimont et al. (16) found four DNA groups, and Farmer et al. (13) identified a fifth DNA group which was composed of isolates from human clinical specimens. Based on an analysis of a few strains, Boemare et al. (6) identified two DNA groups. A conflict between the previously published data and our data involves the affiliation of strain Hm. While Farmer et al. (13) found only 36% DNA similarity between the type strain of *P. luminescens* and strain Hm, the

rDNA sequence data and the DNA reassociation values determined in this study indicate a much higher level of relatedness between these two strains. Certainly the identity of strain Hm distributed to different laboratories should be investigated.

Both rDNA sequencing studies and DNA-DNA reassociation studies confirmed the genomic heterogeneity of *P. luminescens*. As the identity of many host nematodes is unknown, it is not possible at this time to correlate all of the rDNA subclusters (subclusters I to V) with distinct nematode hosts. As shown by the information available, the strains of a defined rDNA subcluster are by and large homogeneous with respect to host species, which may indicate that the symbiotic association is ecologically significant. In conclusion, this study confirms and extends conclusions concerning the phylogenetic diversity of *P. luminescens*, which contains several genospecies. Certainly a search for exclusive subcluster-specific phenotypic properties, which is necessary to circumscribe centers of diversity in a polyphasic approach to classification, is called for.

ACKNOWLEDGMENTS

This study was supported by the U.S.-Hungarian Joint Fund no. 300/91 and the COST 819 action, DEF grant no. 1434.

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