Identification of *Photorhabdus* symbionts by MALDI-TOF mass spectrometry

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Abstract

Species of the bacterial genus *Photorhabus* live in a symbiotic relationship with *Heterorhabditis* entomopathogenic nematodes. Besides their use as biological control agents against agricultural pests, some *Photorhabdus* species are also a source of natural products and are of medical interest due to their ability to cause tissue infections and subcutaneous lesions in humans. Given the diversity of *Photorhabdus* species, rapid and reliable methods to resolve this genus to the species level are needed. In this study, we evaluated the potential of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of *Photorhabdus* species. To this end, we established a collection of 54 isolates consisting of type strains and multiple field strains that belong to each of the validly described species and subspecies of this genus. Reference spectra for the strains were generated and used to complement a currently available database. The extended reference database was then used for identification based on the direct transfer sample preparation method and protein fingerprint of single colonies. High discrimination of distantly related species was observed. However, lower discrimination was observed with some of the most closely related species and subspecies. Our results, therefore, suggest that MALDI-TOF MS can be used to correctly identify *Photorhabdus* strains at the genus and species level, but has limited resolution power for closely related species and subspecies. Our study demonstrates the suitability and limitations of MALDI-TOF-based identification methods for the assessment of the taxonomical position and identification of *Photorhabdus* isolates.

Impact Statement

Species of the bacterial genus *Photorhabus* live in close association with soil-born entomopathogenic nematodes. Under natural conditions, these bacteria are often observed infecting soil-associated arthropods, but under certain circumstances, can also infect humans. They produce a large variety of natural products including antibiotics, insecticides, and polyketide pigments that have substantial agricultural, biotechnological and medical potential. In this study, we implement MALDI-TOF MS-based identification method to resolve the taxonomic identity of this bacterial genus, providing thereby a rapid identification tool to understanding its taxonomic diversity to boost scientific progress in medical, agricultural, and biotechnological settings.

Introduction

Species of the genus *Photorhabdus* live in a close symbiotic association with *Heterorhabditis* entomopathogenic nematodes (1). Given their biosynthetic capacity and ability to produce a large array of specialized metabolites and proteins, and their ability to infect humans and arthropods, *Photorhabdus* species are of biotechnological, medical, and agricultural interest (2–5). Understanding their taxonomic diversity is an important step towards minimizing human health risks and maximizing the agricultural and biotechnological potential of *Photorhabdus* species.

In natural ecosystems, *Photorhabdus* species are carried by entomopathogenic nematodes in their intestines. Entomopathogenic nematodes colonize soil-born arthropods, and release these bacteria in the hemocoel of their prey (6, 7). Bacteria reproduce, produce toxins, immune suppressors, and lytic enzymes, cause septicemia, toxemia, and in many cases kill the infected organism (8). Consequently, these organisms are broadly used as biological control agents to combat agricultural pests (9, 10, 4, 11, 12). Under some particular cases, however, certain *Photorhabdus* species as *Photorhabdus asymbiotica* have been reported to infect humans and cause local tissue infections and subcutaneous nodules (13–16, 3).

Possibly due to their particular lifestyle, *Photorhabdus* species produce an arsenal of secondary metabolites (17–19, 2, 20–22). These metabolites act as virulence factors to kill their prey, symbiosis factors to support the growth of their nematode host, and/or antimicrobial compounds that limit the proliferation of microbial competitors (23–27, 5, 28, 20). Apart from their ecological importance, these metabolites are also valuable for biotechnology. For instance, 3,5-dihydroxy-4-isopropylstilbene, produced by *Photorhabdus* sp. C9, shows antifungal activities against important medical and agricultural fungi like *Aspergillus flavus* and *Candida tropicalis* (29). Another example is carbapenem, an important broad-spectrum β-lactam antibiotic produced by *Photorhabdus luminescence* strain TT01 (30).

Due to their importance as biocontrol agents, human pathogens, and bio-factories, substantial efforts have been made to understand the diversity of the *Photorhabdus* bacterial group. For this, several collection campaigns have been set around the world which have yield many different isolates (31–44). In addition, several methods for the identification of these isolates have been developed and implemented. In medical cases for instance, bacteria isolated from diseased human tissues were identified using classical microbiological methods such as characterization of colony morphology and VITEK 2 Gram-negative identification card-based biochemical tests (45, 15). Unfortunately, these methods misleadingly assigned the causing agent to other bacterial species (46). Routine automated mass spectrometry methods failed to identify the potential disease-causing agent, because *Photorhabdus* spectra were absent from databases. Finally, 16S rRNA gene sequencing had to be performed to properly identify the bacterium that caused the cutaneous lesions (47). Other methods such as restriction fragment length polymorphism-PCR (RFLP-PCR) were used, but also proved to be of very limited taxonomic

value (48, 49). Later, multi locus sequence analysis (MLSA) was found to be a useful tool for the taxonomic description of *Photorhabdus* species (50). However, with an increasing number of available strains and due to the high taxonomic complexity of this bacterial group, whole-genome based methods were shown to be particularly suitable to resolve the phylogenetic relationship of especially closely related *Photorhabdus* species and subspecies (34, 33). Nonetheless, these methods are laborious and do not allow for a rapid identification. The above mentioned limitations might be overcome by MALDI-TOF-based identification techniques (51–55).

In this study, we evaluate the possibility to use MALDI-TOF to rapidly resolve the taxonomic identity of *Photorhabdus* species. To this end, we established an experimental collection of *Photorhabdus* isolates including type and several field strains of all the validly described species of this genus. We then created main spectra libraries and constructed MALDI-TOF MS-based dendrograms. The results of our study highlight the possibilities and limitations of MALDI-TOF MS for the identification of *Photorhabdus* species.

Materials and methods

Bacterial strains

The 54 bacterial strains included in this study were either part of our *in-house* collection, were kindly provided by different collaborators, or were acquired from biological resource centers (Czech Collection of Microorganisms, CCM, or the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, DSMZ). All the bacterial species have been previously isolated from their nematode host or from soft wounds of human patients as described (56, 33, 34, 50, 38–44, 1, 37).

Generation of phylogenetic trees

To identify the *Photorhabdus* bacterial strains as a baseline for the MALDI-TOF experiment, we followed the procedure described by Machado *et. al.* (33, 34). Briefly, bacterial genomic DNA was extracted using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the following primers: 8F AGAGTTTGATCCTGGCTCAG and 1492R CGGTTACCTTGTTACGACTT. PCR products were separated by electrophoresis in a 1 % TAE-agarose gel stained with GelRed nucleic acid gel stain (Biotium), gel-purified (QIAquick gel purification Kit, Qiagen) and sequenced by Sanger sequencing (Microsynth). Obtained sequences were manually curated, trimmed and used to reconstruct evolutionary histories using the Neighbor-Joining method (57). The optimal tree with the sum of branch length = 0.20174092 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (58). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (59) and are in the units of the number of base substitutions per site. There were a total of 1166 positions in the final

105 dataset. Evolutionary analyses were conducted in MEGA7 (60). Graphical representation and edition
106 of the phylogenetic tree were performed with Interactive Tree of Life (version 3.5.1) (61, 62). Whole-
107 genome-based phylogenetic tree was adapted from (34).

108 **Generation of main spectra**

109 Main spectra (MSP) were generated on a microflex™LT (Bruker Daltonik GmbH, Bremen, Germany)
110 as described (63). For this, bacteria were grown from glycerol stocks on Luria Bertani (LB) plates at
111 28°C for 28 hours. Proteins were then extracted following the standard formic acid-based method
112 recommended by the manufacturer (Bruker Daltonik GmbH). Briefly, a few single bacterial colonies
113 were dissolved in 300 µl of pyrogen free water by vortexing and 900 µl of absolute ethanol were added
114 to the solution. After centrifugation (2 min, 15'000 rpm), the supernatant was discarded. After a second
115 centrifugation step and removing the remaining ethanol, the pellet was air dried for 2-3 min. Pellets were
116 then resuspended in 30 µl of 70% formic acid (Sigma Aldrich, Germany). Subsequently, 30 µl of
117 acetonitrile (Fluka analytical, Germany) were added and mixed by pipetting followed by centrifugation
118 for 2 min. at 15'000 rpm. One µl of the resulting supernatant was transferred to the MALDI target plate
119 in eight replicates and let dry at room temperature. Then, 1 µl of matrix (α-Cyano-4-hydroxycinnamic-
120 acid, HCCA, CAS Number 28166-41-8, Sigma-Aldrich, Switzerland) was added. Each spot was then
121 measured in triplicate to obtain 24 spectra per strain using the MBT_AutoX method of flexControl
122 software. The generated spectra were visually inspected and edited in the flexAnalysis software
123 according to Bruker recommendations. Individual spectra diverging from the cohort core, i.e. differing
124 by more than 500 ppm, were deleted. A minimum of 20 spectra per strain were then used for the
125 generation of MSP in the MBT Compass Explorer 4.1 (Bruker) using standard settings. The MSP of
126 each strain was added to the project library used for identification. Newly generated MSP were entered
127 to the project database and were used for identification and to generate a dendrogram using the
128 correlation distance measure with the average linkage algorithm and a threshold value for a single
129 organism of 300 in MBT Compass Explorer 4.1. The MBT Compass Library 4.1 currently contains 3000
130 bacterial species of 540 genera.

131 **Diagnostic identification**

132 To validate the suitability of our newly established main spectra database for the identification of
133 *Photorhabdus* species, several *Photorhabdus* strains with known taxonomic identities were tested. To
134 this end, the bacterial strains were grown for 28 hours at 28°C on LB media. Single colonies were picked
135 with toothpicks, transferred onto the MALDI-TOF target plate, dried at room temperature and mounted
136 with 1 µl of HCCA matrix. Identification of *Photorhabdus* strains was performed by comparing the
137 resulting spectra against the extended Bruker database, including the newly generated *Photorhabdus*
138 MSP.

Results and discussion

A collection of 54 *Photorhabdus* strains belonging to all the 22 validly described species and subspecies was used to evaluate the suitability of MALDI-TOF to identify *Photorhabdus* species. Based on the main spectra produced from the strains, a dendrogram was generated (Figure 1). Two main clusters were observed. All strains of a species clustered together. In some cases, however, clusters composed of strains that belong to two or more species were observed. In particular, all the strains of *P. khanii* subsp. *khanii, P. khanii subsp. guanajuatensis, P. stackebrandtii, P. tasmaniensis*, *P. thracensis*, and *P. thracensis* form a cluster; all strains of *P. cinerea* and *P. heterorhabditis* form a cluster; all strains of *P. luminescens* subsp. *mexicana* and *P. luminescens subsp. luminescens* form a cluster; and all the strains of *P. kayaii, P. kleinii and P. bodei* formed a cluster. The MSP-based dendrogram topology barely resembled the topology of the 16S rRNA gene based phylogenetic tree (Figure 2), but closely mirrored the whole genome-based tree (Figure 3).

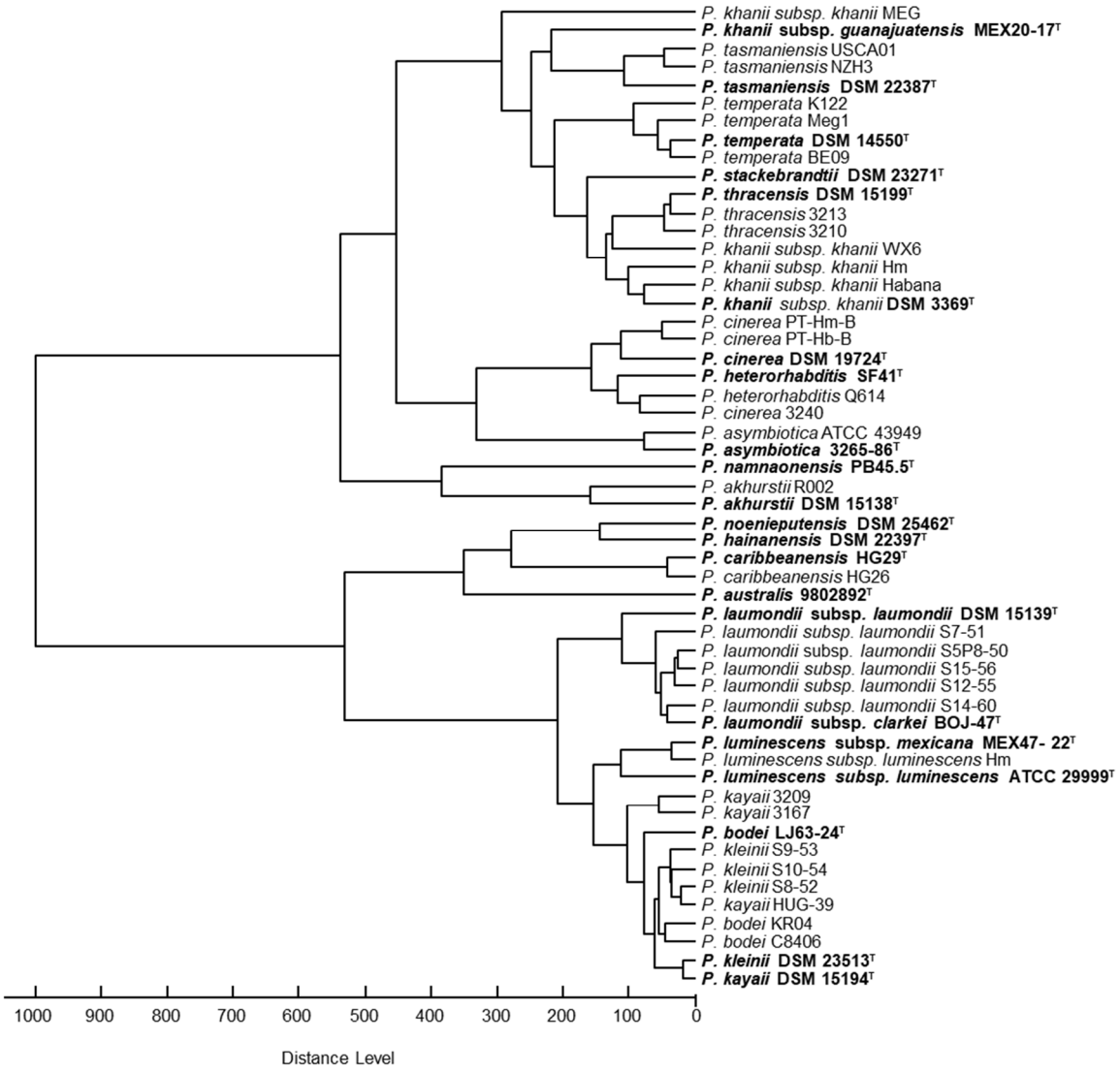

Figure 1 Main spectra (MSP)-based dendrogram of *Photorhabdus* strains. Type strains are indicated in bold. The distance level is normalized to a maximum value of 1000.

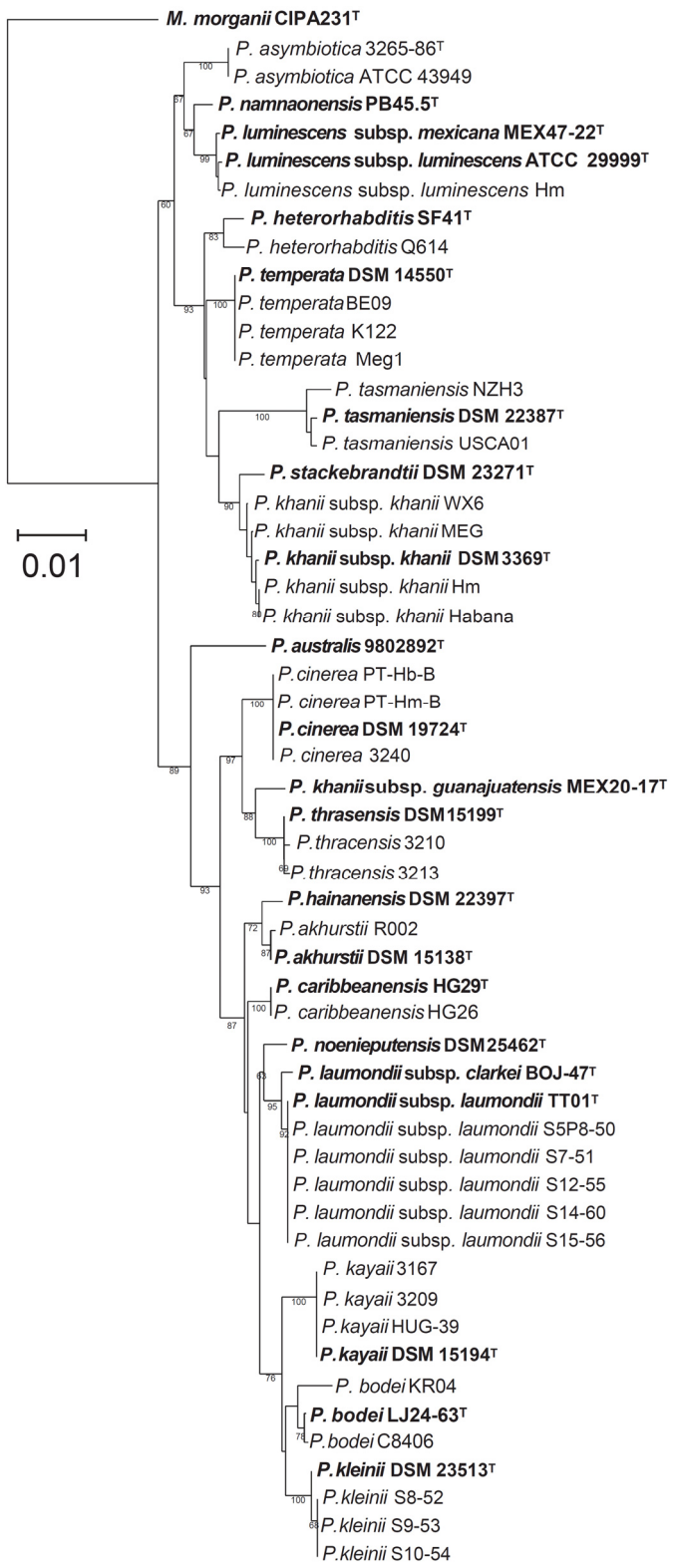

Figure 2 Neighbor-Joining based phylogenetic tree of *Photorhabdus* bacterial strains reconstructed from 1166 nucleotide positions of 16S ribosomal RNA gene sequences. Numbers at nodes represent bootstrap values based on 100 replications. Bar, 0.01 nucleotide substitutions per sequence position. Sequences used were deposited into the National Center for Biotechnology Information (NCBI) databank. Accession numbers are listed in Table S1.

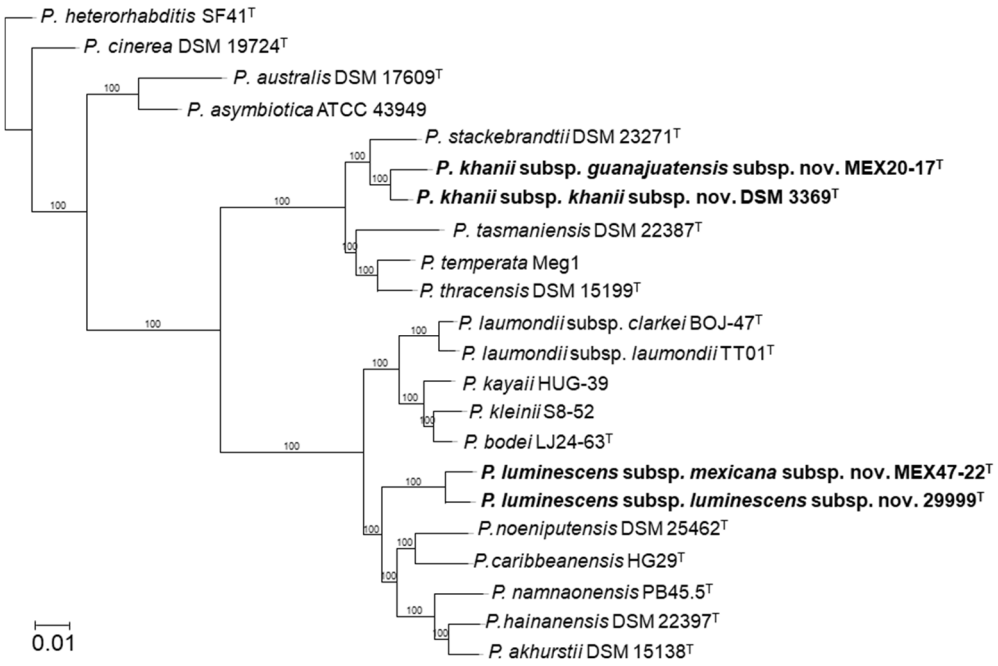

Figure 3 Phylogenetic reconstruction based on core genome sequences of *Photorhabdus* bacterial strains. 1662 open reading frames were analyzed. Numbers at the nodes represent SH-like branch supports. Bar, 0.01 nucleotide substitutions per sequence position.

Using identification scores, all strains could unequivocally be identified up to the genus level, with some limitations for closely related species. Twenty-five (45%) and fifteen (27%) of the analyzed strains appear either first or second, respectively, in the list of strains with best matching scores. For 96% of the strains, greater matching scores were observed with strains of their own species than with members of other species (Table 1). Only two strains *P. laumondii* subsp. *laumondii* S14-60 and *P. namnaonensis* PB45.5^T show similar matching scores with members of other species. We also observed that the identification scores of species that are more closely related, according to whole-genome based phylogenies, tended to be more similar than the scores of strains that are only distantly related. These effects were also observed in the MSP-based dendrogram (Table 1, Figure 1). In particular, we observed that strains of *P. laumondii subsp. laumondii, P. laumondii subsp. clarkei, P. kayaii, P. kleinii and P. bodei* were normally listed within the 10 best scores when analyzing any strain belonging to these species. Similarly occurred for strains that belong to *P. luminescens subsp. luminescens, P. luminescens* subsp. *mexicana, P. noeniputensis, P. caribbeanensis, P. namnaonensis, P. hainanensis* and *P. akhurstii.* For the identification of some strains that belong to the abovementioned species, additional tests might therefore be required. In this context, citrate utilization, indole and acetoin production, and tryptophan deaminase, gelatinase and glucose oxidase activity have been shown to be particularly useful for the discrimination of *Photorhabdus* species (34).

181 **Table 1** MALDI BioTyper identification score values for different *Photorhabdus* strains. Score values
182 higher than 1.99: secure to highly probable species identification; between 1.7 and 1.99: probable genus
183 identification; and between 0.0 and 1.69: not reliable identification. [T] indicates type strain.

Species	Strain	Best match with	
		Same species	Different species
P. akhurstii	DSM 15138[T]	2.26	2.05 (*P. caribbeanensis*)
	R002	2.47	2.18 (*P. hainanensis*)
P. asymbiotica	3265-86[T]	2.38	1.87 (*P. australis*)
	ATCC 43949	2.51	2.06 (*P. australis*)
P. australis	9802892[T]	2.44	1.94 (*P. cinerea*)
P. bodei	LJ63-24[T]	2.54	2.36 (*P. kleinii*)
	KR04	2.38	2.29 (*P. kleinii*)
	C8406	2.46	2.32 (*P. kleinii*)
P. caribbeanensis	HG29[T]	2.62	2.29 (*P. hainanensis*)
	HG26	2.69	2.25 (*P. hainanensis*)
P. cinerea	DSM 19724[T]	2.50	1.80 (*P. asymbiotica*)
	3240	2.63	2.00 (*P. asymbiotica*)
	PT-Hb-B	2.50	1.86 (*P. heterorhabditis*)
	PT-Hm-B	2.55	1.98 (*P. heterorhabditis*)
P. hainanensis	DSM 22397[T]	2.49	2.30 (*P. noenieputensis*)
P. heterorhabditis	SF41[T]	2.37	2.14 (*P. cinerea*)
	Q614	2.46	2.17 (*P. cinerea*)
P. kayaii	DSM 15194[T]	2.26	2.19 (*P. kleinii*)
	HUG-39	2.55	2.39 (*P. bodei*)
	3167	2.51	2.26 (*P. kleinii*)
	3209	2.47	2.37 (*P. kleinii*)
P. khanii subsp. *guanajuatensis*	MEX20-17[T]	2.49	2.17 (*P. thracensis*)
P. khanii subsp. *khanii*	DSM 3369[T]	2.22	1.85 (*P. stackebrandtii*)
	WX6	2.44	2.08 (*P. tasmaniensis*)
	MEG	2.53	2.11 (*P. temperata*)
	Habana	2.53	2.07 (*P. temperata*)
	Hm	2.36	1.88 (*P. thracensis*)
P. kleinii	DSM 23513[T]	2.50	2.44 (*P. bodei*)
	S8-52	2.47	2.32 (*P. bodei*)
	S9-53	2.43	2.33 (*P. bodei*)
	S10-54	2.40	2.37 (*P. bodei*)
P. laumondii subsp. *clarkei*	BOJ-47[T]	2.40	1.88 (*P. luminescens* subsp. *mexicana*)
P. laumondii subsp. *laumondii*	DSM 15139[T]	2.42	2.00 (*P. kayaii*)
	S12-55	2.20	1.77 (*P. kleinii*)
	S5P8-50	2.22	1.70 (*P. kayaii*)
	S15-56	2.50	1.84 (*P. kayaii*)
	S14-60	2.50	2.51 (*P. kleinii*)
	S7-51	2.37	1.86 (*P. kayaii*)
P. luminescens subsp. *luminescens*	ATCC 29999[T]	2.39	2.18 (*P. noenieputensis*)
	Hm	2.44	2.22 (*P. hainaniensis*)
P. luminescens subsp. *mexicana*	MEX47-22[T]	2.56	2.33 (*P. noenieputensis*)
P. namnaonensis	PB45.5[T]	2.33	2.33 (*P. luminescens* subsp. *mexicana*)
P. noenieputensis	DSM 25462[T]	2.53	2.27 (*P. hainanensis*)
P. stackebrandtii	DSM 23271[T]	2.44	2.01 (*P. asymbiotica*)
P. tasmaniensis	DSM 22387[T]	2.53	2.15 (*P. temperata*)
	USCA01	2.37	2.17 (*P. temperata*)
	NZH3	2.44	2.22 (*P. temperata*)
P. temperata	DSM 14550[T]	2.47	2.18 (*P. thracensis*)
	Meg1	2.42	2.11 (*P. tasmaniensis*)
	K122	2.48	2.14 (*P. thracensis*)
	BE09	2.63	2.19 (*P. tasmaniensis*)
P. thracensis	DSM 15199[T]	2.33	2.11 (*P. temperata*)
	3210	2.53	2.28 (*P. temperata*)
	3213	2.49	2.16 (*P. temperata*)

184

185 **Conclusion**

186 MALDI-TOF MS was shown to be a powerful method to identify *Photorhabdus* species at the genus
187 level and in many cases up to the species level. Some limitations were observed for closely related
188 species and subspecies, for which additional tests might be necessary. No special sample preparation is
189 required as the direct transfer sample preparation method is sufficient for generating good quality spectra
190 for comparison against available spectral databases.

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195 Research and Innovation Centre, Hungary), Toyoshi Yoshiga (Saga University, Japan), Waldemar
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201 **Conflicts of interest**

202 The authors declare that there are no conflicts of interest.

203 **Data availability**

204 The MSP generated in this study will be made available to interested scientist upon reasonable request
205 to the authors.

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209 **Supplementary Material**

210 **Table S1.** National Center for Biotechnology Information (NCBI) accession numbers of 16S ribosomal
211 RNA gene sequences of all *Photorhabdus* bacterial strains used in this study.

Species	Strain	NCBI accession number
P. akhurstii	DSM 15138^T	MN714266
	R002	MN714235
P. asymbiotica	3265-86^T	MN714241
	ATCC 43949	MN714278
P. australis	9802892^T	MN714240
P. bodei	LJ63-24^T	MN714238
	KR04	MN714253
	C8406	MN714254
P. caribbeanensis	HG29^T	MN714263
	HG26	MN714279
P. cinerea	DSM 19724^T	MN714242
	3240	MN714273
	PT-Hb-B	MN714231
	PT-Hm-B	MN714232
P. hainanensis	DSM 22397^T	MN714265
P. heterorhabditis	SF41^T	MN714243
	Q614	MN714272
P. kayaii	DSM 15194^T	MN714255
	HUG-39	MN714239
	3167	MN714233
	3209	MN714234
P. khanii subsp. *guanajuatensis*	MEX20-17^T	MN714283
P. khanii subsp. *khanii*	DSM 3369^T	MN714246
	WX6	MN714271
	MEG	MN714269
	Habana	MN714282
	Hm	MN714236
P. kleinii	DSM 23513^T	MN714249
	S8-52	MN714250
	S9-53	MN714251
	S10-54	MN714252
P. laumondii subsp. *clarkei*	BOJ-47^T	MN714237
P. laumondii subsp. *laumondii*	DSM 15139^T	MN714256
	S12-55	MN714259
	S5P8-50	MN714257
	S15-56	MN714261
	S14-60	MN714260
	S7-51	MN714258
P. luminescens subsp. *luminescens*	ATCC 29999^T	MN714262
	Hm	MN714276
P. luminescens subsp. *mexicana*	MEX47-22^T	MN714284
P. namnaonensis	PB45.5^T	MN714267
P. noenieputensis	DSM 25462^T	MN714264
P. stackebrandtii	DSM 23271^T	MN714245
P. tasmaniensis	DSM 22387^T	MN714244
	USCA01	MN714281
	NZH3	MN714280
P. temperata	DSM 14550^T	MN714247
	Meg1	MN714277
	K122	MN714275
	BE09	MN714274
P. thracensis	DSM 15199^T	MN714248
	3210	MN714268
	3213	MN714270

212 **References**

213 1. Hazir S, Kaya HK, Stock SP, Keskin N. 2004. Entomopathogenic nematodes (Steinernematidae
214 and Heterorhabditidae) for biological control of soil pests. Turkish Journal of Biology 27:181–202.
215 2. Bode HB. 2009. Entomopathogenic bacteria as a source of secondary metabolites. Current opinion
216 in chemical biology 13:224–230.
217 3. Gerrard J, Waterfield N, Vohra R. 2004. Human infection with *Photorhabdus asymbiotica*: an
218 emerging bacterial pathogen. Microbes and Infection 6:229–237.
219 4. Smart GC. 1995. Entomopathogenic nematodes for the biological control of insects. J Nematol
220 27:529.
221 5. Dowling A, Waterfield NR. 2007. Insecticidal toxins from *Photorhabdus* bacteria and their
222 potential use in agriculture. Toxicon 49:436–451.
223 6. Ciche TA, Kim K-s, Kaufmann-Daszczuk B, Nguyen KCQ, Hall DH. 2008. Cell invasion and
224 matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora*
225 nematodes. Appl Environ Microbiol 74:2275–2287.
226 7. Zhang X, Machado RAR, van Doan C, Arce CCM, Hu L, Am Robert C. 2019. Entomopathogenic
227 nematodes increase predation success by inducing cadaver volatiles that attract healthy herbivores.
228 eLife 8.
229 8. Gerdes E, Upadhyay D, Mandjiny S, Bullard-Dillard R, Storms M, Menefee M, Holmes LD. 2015.
230 *Photorhabdus luminescens*: virulent properties and agricultural applications. American Journal of
231 Agriculture and Forestry 3:171–177.
232 9. Waterfield NR, Ciche T, Clarke D. 2009. *Photorhabdus* and a host of hosts. Annu. Rev. Microbiol.
233 63:557–574. doi:10.1146/annurev.micro.091208.073507.
234 10. Lacey LA, Georgis R. 2012. Entomopathogenic nematodes for control of insect pests above and
235 below ground with comments on commercial production. J Nematol 44:218–225.
236 11. Jackson JJ. 1996. Field performance of entomopathogenic nematodes for suppression of western
237 corn rootworm (Coleoptera: Chrysomelidae). Journal of economic entomology 89:366–372.
238 12. Hiltpold I. 2015. Prospects in the application technology and formulation of entomopathogenic
239 nematodes for biological control of insect pests, p. 187–205. *In* Nematode pathogenesis of insects
240 and other pests. Springer.
241 13. Costa SCP, Girard PA, Brehelin M, Zumbihl R. 2009. The emerging human pathogen
242 *Photorhabdus asymbiotica* is a facultative intracellular bacterium and induces apoptosis of
243 macrophage-like cells. Infection and Immunity 77:1022–1030. doi:10.1128/IAI.01064-08.
244 14. Hapeshi A, Waterfield NR. 2016. *Photorhabdus asymbiotica* as an insect and human pathogen, p.
245 159–177. *In* The Molecular Biology of *Photorhabdus* Bacteria. Springer.
246 15. Weissfeld AS, Halliday RJ, Simmons DE, Trevino EA, Vance PH, O'Hara CM, Sowers EG, Kern
247 R, Koy RD, Hodde K. 2005. *Photorhabdus asymbiotica*, a pathogen emerging on two continents
248 that proves that there is no substitute for a well-trained clinical microbiologist. Journal of clinical
249 microbiology 43:4152–4155.
250 16. Gerrard JG, Joyce SA, Clarke DJ. 2006. Nematode symbiont for *Photorhabdus asymbiotica*.
251 Emerging infectious diseases 12:1562.
252 17. Kontnik R, Crawford JM, Clardy J. 2010. Exploiting a global regulator for small molecule
253 discovery in *Photorhabdus luminescens*. ACS chemical biology 5:659–665.
254 18. Joyce SA, Lango L, Clarke DJ. 2011. The regulation of secondary metabolism and mutualism in
255 the insect pathogenic bacterium *Photorhabdus luminescens*, p. 1–25. *In* Advances in applied
256 microbiology, vol. 76. Elsevier.
257 19. Clarke DJ. 2008. *Photorhabdus*: a model for the analysis of pathogenicity and mutualism. Cellular
258 microbiology 10:2159–2167.
259 20. Tobias NJ, Heinrich AK, Eresmann H, Wright PR, Neubacher N, Backofen R, Bode HB. 2017.
260 *Photorhabdus*-nematode symbiosis is dependent on hfq-mediated regulation of secondary
261 metabolites. Environ Microbiol 19:119–129. doi:10.1111/1462-2920.13502.

21. Joyce SA, Brachmann AO, Glazer I, Lango L, Schwär G, Clarke DJ, Bode HB. 2008. Bacterial biosynthesis of a multipotent stilbene. Angewandte Chemie International Edition 47:1942–1945.
22. Kushwah J, Somvanshi VS. 2015. *Photorhabdus*: a microbial factory of insect-killing toxins, p. 235–240. *In* Microbial Factories. Springer.
23. Campos-Herrera R, Jaffuel G, Chiriboga X, Blanco-Pérez R, Fesselet M, Půža V, Mascher F, Turlings TCJ. 2015. Traditional and molecular detection methods reveal intense interguild competition and other multitrophic interactions associated with native entomopathogenic nematodes in Swiss tillage soils. Plant and Soil 389:237–255.
24. Campos-Herrera R, Půža V, Jaffuel G, Blanco-Pérez R, Čepulytė-Rakauskienė R, Turlings TCJ. 2015. Unraveling the intraguild competition between *Oscheius* spp. nematodes and entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils. Journal of invertebrate pathology 132:216–227.
25. Joyce SA, Watson RJ, Clarke DJ. 2006. The regulation of pathogenicity and mutualism in *Photorhabdus*. Current opinion in microbiology 9:127–132.
26. Strauch O, Ehlers R-U. 1998. Food signal production of *Photorhabdus luminescens* inducing the recovery of entomopathogenic nematodes *Heterorhabditis* spp. in liquid culture. Applied microbiology and biotechnology 50:369–374.
27. Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R. 1998. Insecticidal toxins from the bacterium *Photorhabdus luminescens*. Science 280:2129–2132.
28. Lang AE, Schmidt G, Schlosser A, Hey TD, Larrinua IM, Sheets JJ, Mannherz HG, Aktories K. 2010. *Photorhabdus luminescens* toxins ADP-ribosylate actin and RhoA to force actin clustering. Science 327:1139–1142.
29. Li J, Chen G, Wu H, Webster JM. 1995. Identification of two pigments and a hydroxystilbene antibiotic from *Photorhabdus luminescens*. Appl Environ Microbiol 61:4329–4333.
30. Derzelle S, Duchaud E, Kunst F, Danchin A, Bertin P. 2002. Identification, characterization, and regulation of a cluster of genes involved in carbapenem biosynthesis in *Photorhabdus luminescens*. Appl Environ Microbiol 68:3780–3789.
31. Thanwisai A, Tandhavanant S, Saiprom N, Waterfield NR, Long PK, Bode HB, Peacock SJ, Chantratita N. 2012. Diversity of *Xenorhabdus* and *Photorhabdus* spp. and their symbiotic entomopathogenic nematodes from Thailand. PloS one 7:e43835.
32. Zhang X, van Doan C, Arce CCM, Hu L, Gruenig S, Parisod C, Hibbard BE, Hervé MR, Nielson C, Am Robert C. 2019. Plant defense resistance in natural enemies of a specialist insect herbivore. Proceedings of the National Academy of Sciences 116:23174–23181.
33. Machado RAR, Wüthrich D, Kuhnert P, Arce CCM, Thönen L, Ruiz C, Zhang X, Robert CAM, Karimi J, Kamali S, Ma J, Bruggmann R, Erb M. 2018. Whole-genome-based revisit of *Photorhabdus* phylogeny: proposal for the elevation of most *Photorhabdus* subspecies to the species level and description of one novel species *Photorhabdus bodei* sp. nov., and one novel subspecies *Photorhabdus laumondii* subsp. *clarkei* subsp. nov. International Journal of Systematic and Evolutionary Microbiology 68:2664–2681. doi:10.1099/ijsem.0.002820.
34. Machado RAR, Bruno P, Arce CCM, Liechti N, Köhler A, Bernal J, Bruggmann R, Turlings TCJ. 2019. *Photorhabdus khanii* subsp. *guanajuatensis* subsp. nov., isolated from *Heterorhabditis atacamensis*, and *Photorhabdus luminescens* subsp. *mexicana* subsp. nov., isolated from *Heterorhabditis mexicana* entomopathogenic nematodes. International Journal of Systematic and Evolutionary Microbiology 69:652–661. doi:10.1099/ijsem.0.003154.
35. Thomas GM, Poinar G. 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. International Journal of Systematic and Evolutionary Microbiology 29:352–360.
36. Tóth T, Lakatos T. 2008. *Photorhabdus temperata* subsp. *cinerea* subsp. nov., isolated from *Heterorhabditis* nematodes. International Journal of Systematic and Evolutionary Microbiology 58:2579–2581.
37. Szallas E, Koch C, Fodor A, Burghardt J, Buss O, Szentirmai A, Nealson KH, Stackebrandt E. 1997. Phylogenetic evidence for the taxonomic heterogeneity of *Photorhabdus luminescens*. International Journal of Systematic and Evolutionary Microbiology 47:402–407.

38. Hazir S, Stackebrandt E, Lang E, Schumann P, Ehlers R-U, Keskin N. 2004. Two new Subspecies of *Photorhabdus luminescens*, Isolated from *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae): *Photorhabdus luminescens* subsp. *kayaii* subsp. nov. and *Photorhabdus luminescens* subsp. *thracensis* subsp. nov. Systematic and applied microbiology 27:36–42.
39. Fischer-Le Saux M, Viallard V, Brunel B, Normand P, Boemare NE. 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. International Journal of Systematic and Evolutionary Microbiology 49:1645–1656.
40. Boemare NE, Akhurst RJ, Mourant RG. 1993. DNA relatedness between *Xenorhabdus* spp.(Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. International Journal of Systematic and Evolutionary Microbiology 43:249–255.
41. An R, Grewal PS. 2011. *Photorhabdus luminescens* subsp. *kleinii* subsp. nov.(Enterobacteriales: Enterobacteriaceae). Current microbiology 62:539–543.
42. An R, Grewal PS. 2010. *Photorhabdus temperata* subsp. *stackebrandtii* subsp. nov.(Enterobacteriales: Enterobacteriaceae). Current microbiology 61:291–297.
43. Akhurst RJ, Boemare NE, Janssen PH, Peel MM, Alfredson DA, Beard CE. 2004. Taxonomy of Australian clinical isolates of the genus *Photorhabdus* and proposal of *Photorhabdus asymbiotica* subsp. *asymbiotica* subsp. nov. and *P. asymbiotica* subsp. *australis* subsp. nov. International Journal of Systematic and Evolutionary Microbiology 54:1301–1310.
44. Ferreira T, van Reenen C, Pages S, Tailliez P, Malan AP, Dicks LMT. 2013. *Photorhabdus luminescens* subsp. *noenieputensis* subsp. nov., a symbiotic bacterium associated with a novel *Heterorhabditis* species related to *Heterorhabditis indica*. International Journal of Systematic and Evolutionary Microbiology 63:1853–1858.
45. Gerrard JG, Stevens RP. 2017. A review of clinical cases of infection with *Photorhabdus asymbiotica*, p. 179–191. *In* ffrench-Constant RH (ed), The molecular biology of *Photorhabdus* bacteria, vol. 402. Springer International Publishing, Cham.
46. Gerrard JG, Vohra R, Nimmo GR. 2003. Identification of *Photorhabdus asymbiotica* in cases of human infection. Communicable diseases intelligence quarterly report 27:540.
47. Dutta A, Flores AR, Revell PA, Owens L. 2018. Neonatal bacteremia and cutaneous lesions caused by *Photorhabdus luminescens*: A rare Gram-negative bioluminescent bacterium. J Pediatric Infect Dis Soc 7:e182-e184. doi:10.1093/jpids/piy064.
48. Brunel B, Givaudan A, Lanois A, Akhurst RJ, Boemare N. 1997. Fast and accurate identification of *Xenorhabdus* and *Photorhabdus* species by restriction analysis of PCR-amplified 16S rRNA genes. Appl Environ Microbiol 63:574–580.
49. Peel MM, Alfredson DA, Gerrard JG, Davis JM, Robson JM, McDougall RJ, Scullie BL, Akhurst RJ. 1999. Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. Journal of clinical microbiology 37:3647–3653.
50. Glaeser SP, Tobias NJ, Thanwisai A, Chantratita N, Bode HB, Kämpfer P. 2017. *Photorhabdus luminescens* subsp. *namnaonensis* subsp. nov., isolated from *Heterorhabditis baujardi* nematodes. International Journal of Systematic and Evolutionary Microbiology 67:1046–1051. doi:10.1099/ijsem.0.001761.
51. Vargha M, Takáts Z, Konopka A, Nakatsu CH. 2006. Optimization of MALDI-TOF MS for strain level differentiation of Arthrobacter isolates. Journal of Microbiological Methods 66:399–409.
52. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, Raoult D. 2009. Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. CLIN INFECT DIS 49:543–551. doi:10.1086/600885.
53. Sauer S, Kliem M. 2010. Mass spectrometry tools for the classification and identification of bacteria. Nat Rev Microbiol 8:74–82. doi:10.1038/nrmicro2243.

54. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti J-L, Ferroni A, Gutmann L, Nassif X. 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clinical Biochemistry 44:104–109. doi:10.1016/j.clinbiochem.2010.06.017.
55. Maier T, Klepel S, Renner U, Kostrzewa M. 2006. Fast and reliable maldi-tof ms–based microorganism identification. Nature Methods 3:i–ii.
56. Tailliez P, Laroui C, Ginibre N, Paule A, Pagès S, Boemare N. 2010. Phylogeny of *Photorhabdus* and *Xenorhabdus* based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa: *X. vietnamensis* sp. nov., P. luminescens subsp. caribbeanensis subsp. nov., P. luminescens subsp. hainanensis subsp. nov., P. temperata subsp. khanii subsp. nov., P. temperata subsp. tasmaniensis subsp. nov., and the reclassification of *P. luminescens* subsp. *thracensis* as *P. temperata* subsp. *thracensis* comb. nov. International Journal of Systematic and Evolutionary Microbiology 60:1921–1937. doi:10.1099/ijs.0.014308-0.
57. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.
58. Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783. doi:10.2307/2408678.
59. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. doi:10.1007/BF01731581.
60. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870–1874. doi:10.1093/molbev/msw054.
61. Chevenet F, Brun C, Bañuls A-L, Jacq B, Christen R. 2006. TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinformatics 7:357. doi:10.1186/1471-2105-7-439.
62. Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 44:W242-W245. doi:10.1093/nar/gkw290.
63. Kuhnert P, Bisgaard M, Korczak BM, Schwendener S, Christensen H, Frey J. 2012. Identification of animal Pasteurellaceae by MALDI-TOF mass spectrometry. Journal of Microbiological Methods 89:1–7. doi:10.1016/j.mimet.2012.02.001.