Photorhabdus heterorhabditis subsp. aluminescens subsp. nov., Photorhabdus heterorhabditis subsp. heterorhabditissubsp. nov., Photorhabdus australis subsp. thailandensis subsp. nov., Photorhabdus australis subsp. australis subsp. nov., and Photorhabdus aegyptia sp. nov. isolated from Heterorhabditis entomopathogenic nematodes

Ricardo Machado, Arthur Muller, Shima Ghazal, Aunchalee Thanwisai, Sylvie Pages, Helge Bode, Mona Hussein, Kamal Khalil, Louis Tisa

To cite this version:

Ricardo Machado, Arthur Muller, Shima Ghazal, Aunchalee Thanwisai, Sylvie Pages, et al.. Photorhabdus heterorhabditis subsp. aluminescens subsp. nov., Photorhabdus heterorhabditis subsp. heterorhabditissubsp. nov., Photorhabdus australis subsp. thailandensis subsp. nov., Photorhabdus australis subsp. australis subsp. nov., and Photorhabdus aegyptia sp. nov. isolated from Heterorhabditis entomopathogenic nematodes. International Journal of Systematic and Evolutionary Microbiology, Microbiology Society, 2021, 71 (1), 10.1099/ijsem.0.004610. hal-03143425

HAL Id: hal-03143425
https://hal.inrae.fr/hal-03143425
Submitted on 16 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Photorhabdus heterorhabditis subsp. aluminescens subsp. nov., Photorhabdus heterorhabditis subsp. heterorhabditis subsp. nov., Photorhabdus australis subsp. thailandensis subsp. nov., Photorhabdus australis subsp. australis subsp. nov., and Photorhabdus aegyptia sp. nov. isolated from Heterorhabditis entomopathogenic nematodes

Ricardo A. R. Machado1,*, Arthur Muller1, Shima M. Ghazal2,3, Aunchalee Thanwisai4, Sylvie Pagès5, Helge B. Bode6, Mona A. Hussein7, Kamal M. Khalil3 and Louis S. Tisa8

Abstract

Three Gram-stain-negative, rod-shaped, non-spore-forming bacteria, BA1T, Q614T and PB68.1T, isolated from the digestive system of Heterorhabditis entomopathogenic nematodes, were biochemically and molecularly characterized to clarify their taxonomic affiliations. The 16S rRNA gene sequences of these strains suggest that they belong to the Gammaproteobacteria, to the family Morganellaceae, and to the genus Photorhabdus. Deeper analyses using whole genome-based phylogenetic reconstructions suggest that BA1T is closely related to Photorhabdus akhursti, that Q614T is closely related to Photorhabdus heterorhabditis, and that PB68.1T is closely related to Photorhabdus australis. In silico genomic comparisons confirm these observations: BA1T and P. akhursti 15138T share 68.8% digital DNA–DNA hybridization (dDDH), Q614T and P. heterorhabditis SF41T share 75.4% dDDH, and PB68.1T and P. australis DSM 17609T share 76.6% dDDH. Physiological and biochemical characterizations reveal that these three strains also differ from all validly described Photorhabdus species and from their more closely related taxa, contrary to what was previously suggested. We therefore propose to classify BA1T as a new species within the genus Photorhabdus, Q614T as a new subspecies within P. heterorhabditis, and PB68.1T as a new subspecies within P. australis. Hence, the following names are proposed for these strains: Photorhabdus aegyptia sp. nov. with the type strain BA1T (=DSM 111180T=CCOS 1943T=LMG 31957T), Photorhabdus heterorhabditis subsp. aluminescens subsp. nov. with the type strain Q614T (=DSM 111144T=CCOS 1944T=LMG 31959T) and Photorhabdus australis subsp. thailandensis subsp. nov. with the type strain PB68.1T (=DSM 111145T=CCOS 1942T). These propositions automatically create Photorhabdus heterorhabditis subsp. heterorhabditis subsp. nov. with SF41T as the type strain (currently classified as P. heterorhabditis) and Photorhabdus australis subsp. australis subsp. nov. with DSM17609T as the type strain (currently classified as P. australis).

Species of the bacterial genus Photorhabdus live in a close symbiotic relationship with Heterorhabditis entomopathogenic nematodes (EPNs) [1]. EPNs are soil-inhabiting organisms that parasitize and reproduce inside small arthropods [2, 3]. They colonize their prey by penetrating through the cuticle or natural openings such as the mouth, spiracles

Author affiliations: 1Experimental Biology Research Group, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland; 2Department of Biological Sciences, University of New Hampshire, Durham, New Hampshire, USA; 3Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Center, Cairo, Egypt; 4Department of Microbiology and Parasitology, Faculty of Medical Science, Onusar University, Phitsanulok, Thailand; 5INRAe, Université de Montpellier, UMR1333-DGIMI, 34095 Montpellier Cedex 05, France; 6Molekulare Biotechnologie, Fachbereich Biowissenschaften & Buchmann Institute for Molecular Life Sciences (BMLS), Goethe-Universität Frankfurt am Main & Senckenberg Gesellschaft für Naturforschung, Frankfurt, Germany; 7Department of Pests and Plant Protection, Agricultural and Biological Division, National Research Centre, Dokki, Cairo, Egypt; 8Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, New Hampshire, USA.

*Correspondence: Ricardo A. R. Machado, richardo.machado@unine.ch

Keywords: dDDH; Heterorhabditis; Photorhabdus; polyphasic classification; whole genome sequencing.

Whole genome sequences are deposited in the National Center for Biotechnology Information (NCBI) databank under the following accession numbers: BA1T (JFGV01), Q614T (JABBCS01), and PB68.1T (LOMY01). 16S rRNA gene sequences were also deposited in the NCBI databank under the following accession numbers: BA1T (MT355495), Q614T (AY216500.1), and PB68.1T (MT355494).

One supplementary table is available with the online version of this article.
or the anus, and crawl towards the haemocoel where they release their *Photorhabdus* symbiotic bacterial partners [4]. *Photorhabdus* bacteria multiply, produce immunosuppressors, digestive proteins and secondary metabolites that cause toxemia, sepsis and eventually kill the infected organism [1, 5–8]. The genus *Photorhabdus* was described by Boemare *et al.* in 1993 to include symbiotic bacteria of *Heterorhabditis* EPNs [9]. Since then, several species and subspecies have been described [9–24]. Currently, the genus *Photorhabdus* contains the following 19 species with validly published names: *Photorhabdus akhurstii*, *Photorhabdus asymbiotica*, *Photorhabdus australis*, *Photorhabdus bodei*, *Photorhabdus caribbeanensis*, *Photorhabdus cinerea*, *Photorhabdus hainanensis*, *Photorhabdus heterorhabditis*, *Photorhabdus kaiutii*, *Photorhabdus khanii*, *Photorhabdus kleinii*, *Photorhabdus laumondii*, *Photorhabdus luminescens*, *Photorhabdus namnaonensis*, *Photorhabdus noenieputensis*, *Photorhabdus stackebrandti*, *Photorhabdus tasmaniensis*, *Photorhabdus temperata* and *Photorhabdus thracensis*. *Photorhabdus laumondii* is divided into two subspecies: *P. laumondii subsp. laumondii* and *P. laumondii subsp. clarkei*; *Photorhabdus khanii* is divided into two subspecies: *P. khanii subsp. khanii* and *P. khanii subsp. guanajuatensis*; and *Photorhabdus luminescens* is divided into two subspecies: *P. luminescens subsp. luminescens* and *P. luminescens subsp. mexicana* [16, 17].

Through the years, the taxonomy and nomenclature of this bacterial group have undergone profound changes as more strains have been isolated and new technological capabilities to characterize them have emerged [9, 13, 17, 18, 21]. As a result, the taxonomy of *Photorhabdus* is apparently clearer and whether a bacterial strain represents a new taxon or not is more straightforward to determine than in the past. However, there are still several taxonomic uncertainties, especially regarding bacterial strains that are highly similar to the type strain of the species that they were circumscribed or that were not formally characterized at the taxonomic level. This is the case of at least three bacterial strains: BA1, PB68.1 and Q614 [12, 25–28]. They were isolated from the intestines of *Heterorhabditis* EPNs that were recovered from soil samples in Egypt, Thailand and Australia, respectively. These strains were initially classified as *P. luminescens* BA1, *P. australis* subsp. *australis* PB68.1 and *Xenorhabdus luminescens* Q614. Based on the most recent taxonomy and nomenclature of this bacterial group, these strains are currently classified as *P. luminescens* BA1, *P. australis* subsp. *australis* PB68.1 and *P. heterorhabditis* Q614. This classification relies to a great extent on 16S rRNA and/or housekeeping gene sequences. As it recently became evident that housekeeping gene and 16S rRNA gene sequences provide insufficient information to resolve the phylogenetic relationships of this bacterial group, particularly of closely related taxa, we revisited the phylogenetic relationships of these strains based on whole genome sequences [16, 17]. To this end, we conducted whole genome-based phylogenetic analyses and sequence comparative studies and found out that these three strains actually represent new taxa within the genus *Photorhabdus*. Here we characterize these strains to describe these new taxa and present our taxonomic conclusions.

To physiologically, biochemically and morphologically characterize strains BA1, PB68.1 and Q614, we used bacterial cultures from a single primary form colony of each strain. Bacteria primary forms were determined by examining colony characteristics on NBTA plates [Luria–Bertani (LB) agar plates supplemented with 25 mg ml⁻¹ bromothymol blue and 4 mg ml⁻¹ triphenyl-2,3,5-tetrazolium chloride]. The selected primary form colony was further sub-cultured and maintained on LB agar plates at 28°C. Cell morphology was observed under a Zeiss light microscope at a magnification of ×1000, with cells grown for 5 days at 28°C on LB agar plates. Motility was tested on soft agar as described [29]. Catalase activity was determined by adding a drop of 10% (v/v) H₂O₂ into 50 μl of a liquid LB-grown, 24-h-old bacterial culture. The ability of bacterial strains to absorb dye was tested by growing the cells on NBTA agar containing bromothymol blue and triphenyl-2,3,5-tetrazolium chloride (Sigma–Aldrich). Catalase activity was determined by adding a drop of 10% (v/v) H₂O₂ into 50 μl of a liquid LB-grown, 24-h-old bacterial culture. The ability of bacterial strains to absorb dye was tested by growing the cells on NBTA agar containing bromothymol blue and triphenyl-2,3,5-tetrazolium chloride (Sigma–Aldrich) [30]. Bioluminescence was determined from liquid cultures using a TriStar LB 942 Multimode Microplate Reader (Berthold Technologies). API 20E strips were used according to manufacturer’s instructions (BioMérieux). In this case, strains BA1, Q614 and PB68.1 were tested in parallel and the results obtained were compared to those published previously and obtained using all *Photorhabdus* type strains [16].

To molecularly characterize strains BA1, Q614 and PB68.1, we reconstructed phylogenetic relationships based on 16S rRNA gene sequences and whole genome sequences, and calculated sequence similarity scores. As the full genome sequences of certain type strains are not publicly available, the set of strains used to reconstruct phylogenetic relationships based on 16S rRNA gene sequences, whole genome sequences and biochemical tests are slightly different. Genome sequences of BA1, Q614 and PB68.1 were obtained as described previously [6, 16, 17, 25]. Whole genome sequence similarities were calculated by the digital DNA–DNA hybridization (dDDH) method using formula 2 of the Genome-to-Genome Distance Calculator (GGDC) web service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) [31–34]. Whole genome-based phylogenetic relationships were reconstructed using the Reference sequence Alignment based Phylogeny builder realphy 1.12 and FastTree 2.1.10 [35–39]. 16S rRNA genes were amplified by PCR and sequenced by Sanger sequencing [40, 41]. The 16S rRNA gene-based phylogenetic relationships were reconstructed using the maximum-likelihood method based on the Kimura two-parameter model in MEGA7 [42, 43]. Sequences were aligned with MUSCLE (version 3.8.31) [44]. The tree with the highest log likelihood (−3554.61) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach.
Given the high 16S rRNA gene sequence similarity scores observed and that 16S rRNA and housekeeping gene sequences provide insufficient information to resolve the phylogenetic relationships of this bacterial group, particularly of very closely related species [16, 17], and to fully meet the guidelines of the ad hoc Committee on Reconciliation of Approaches to Bacterial Systematics [47] that recommends the dDDH method as the gold standard for bacterial species circumscription, we reconstructed phylogenetic trees based on core genomes and calculated dDDH scores to determine the taxonomic position of these strains. Strain BA1T clusters together with *P. akhurstii* DSM 15138T and *P. hainanensis* DSM 22397T, Q614T clusters together with *P. heterorhabditis* SF41T, and PB68.1T clusters together with *P. australis* DSM 17609T (Fig. 3). The dDDH scores between BA1T and all *Photorhabdus* species were lower than 70% (Fig. 4). The dDDH scores between Q614T and all *Photorhabdus* species, except *P. heterorhabditis* SF41T, were lower than 70%. The dDDH scores between PB68.1T and all *Photorhabdus* species, except *P. australis* DSM 17609T, were lower than 70%. Strains Q614T and *P. heterorhabditis* SF41T share 75.4% dDDH (CI: 72.4–78.1%), and strains PB68.1T and *P. australis* DSM 17609T share 76.6% dDDH (CI: 73.6–79.4%). Given that the thresholds for species and subspecies delimitation are 70% and 79% dDDH, respectively, we propose to classify BA1T as a new *Photorhabdus* species, Q614T as a new subspecies within the species *P. heterorhabditis* and PB68.1T as a new subspecies within the species *P. australis* [16, 32, 47]. Biochemical characterization supports the status of BA1T, Q614T and PB68.1T as new taxa.
since they exhibit unique biochemical profiles, which differ from the profiles of other strains from other taxa (Table 1). Citrate utilization, acetoin and indole production, and urease activity are particularly suitable biochemical tests to differentiate the different species of the genus Photorhabdus (Table 1).

Based on the results of this polyphasic approach, we propose the creation of Photorhabdus aegyptia sp. nov. with the type strain BA1T (=DSM 111180T=CCOS 1943T=LMG 31957T), Photorhabdus heterorhabditis subsp. aluminescens subsp. nov. with the type strain Q614T (=DSM 111144T=CCOS 1944T=LMG 31959T) and Photorhabdus australis subsp. thailandensis subsp. nov. with the type strain PB68.1T (=DSM 111145T=CCOS 1942T). These propositions automatically create Photorhabdus heterorhabditis subsp. heterorhabditis subsp. nov. BA1T and Photorhabdus australis subsp. australis subsp. nov. with DSM 17609T as the type strain (currently classified as P. australis).

**EMENDED DESCRIPTION OF PHOTORHABDUS AUSTRALIS (AKHURST ET AL. 2004)**

**MACHADO ET AL. 2018**

Photorhabdus australis (aus. tra’lis. L. fem. adj. australis: southern; the type strain of this species was detected in the southern hemisphere).

Maximum temperature for growth is 40°C. Yellow or no pigment; weakly pigmented. Most isolates are positive for DNase and most are negative for aesculin hydrolysis. Negative for urease and indole production. Most isolates produce acid from gluconate, variable for acid production from aesculin and negative for trehalose. Proteinaceous inclusions are rare. β-Galactosidase weak or positive. Annular haemolysis is variable on sheep blood and horse blood agars. Most isolates are negative for Tween 60 and Tween 80 esterases and most grow on myo-inositol. Natural habitat is Heterorhabditis EPNs; all isolates were obtained from human clinical specimens in Australia. The type strain of the species is 9802892T (=CIP 108025T=ACM 5210T).
DESCRIPTION OF PHOTORHABDUS AUSTRALIS SUBSP. AUSTRALIS SUBSP. NOV.

Photorhabdus australis subsp. australis (australis L. fem. adj. australis: southern; the type strain of the species was detected in the Southern Hemisphere).

Maximum temperature for growth is 40 °C. Yellow or no pigment; weakly pigmented. Most isolates are positive for DNase and most are negative for aesculin hydrolysis. Negative for urease and indole production. Most isolates produce acid from gluconate; variable for acid production from aesculin and negative for trehalose. Proteinaceous inclusions are rare. Weak β-galactosidase activity. Annular haemolysis is variable on sheep blood and horse blood agars. The type strain of the species was detected in the Southern Hemisphere.

EMENDED DESCRIPTION OF PHOTORHABDUS HETERORHABDITIS FERREIRA ET AL. 2014

Photorhabdus heterorhabditis (he.te.ro.rhab'd.it.is. N.L. gen. n. heterorhabditis of the nematode Heterorhabditis).

Cells are Gram-stain-negative, catalase-positive rods. Bioluminescence variable. Aerobic growth is preferred, with growth temperatures ranging from 24 to 42 °C in nutrient broth (NB) and from 24 to 35 °C in tryptic soy broth (TSB). Optimal growth in NB and TSB occurs at 30 °C. Colonies on NBTA are blue or blue-green. Indole production negative. Citrate utilization variable. Urease variable. Tryptophan deaminase variable. Acid is produced from N-acetylglucosamine, d-fructose, d-glucose, glycerol, d-mannose, maltose and d-xylose. Able to ferment glucose, hydrolyse arginine, aesculin and gelatin, and produce urease. Assimilates glucose, d-mannose, N-acetylglucosamine, maltose and potassium gluconate (weakly). Nitrate is not reduced. This strain was isolated from Heterorhabditis...
zealandica EPNs collected in South Africa. The type strain of the species is SF41<sup>T</sup> (=ATCC BAA-2479<sup>T</sup>=DSM 25263<sup>T</sup>).

**DESCRIPTION OF PHOTORHABDUS HETERORHABDITIS SUBSP. HETERORHABDITIS SUBSP. NOV.**

*Photorhabdus heterorhabditis* subsp. *heterorhabditis* (he. ro. rhab′di.tis. N.L. gen. n. *heterorhabditis* of the nematode *Heterorhabditis*).

Cells are Gram-stain-negative, catalase-positive rods. Bioluminescent. Aerobic growth is preferred, with growth temperatures ranging from 24 to 42 °C in NB and from 24 to 35 °C in TSB. Optimal growth in NB and TSB occurs at 30 °C. Colonies on NBTA are blue or blue-green. Acid is produced from *N*-acetylglucosamine, *d*-fructose, *d*-glucose, glycerol, *d*-mannose, maltose and *d*-xylose. Able to ferment glucose, hydrolyse arginine, aesculin and gelatin, and produce urease. Assimilates glucose, *d*-mannose, *N*-acetylglucosamine, maltose and potassium gluconate (weakly). Indole production negative. Citrate utilization negative. Urease negative. Tryptophan deaminase positive. Nitrate is not reduced. This strain was isolated from *Heterorhabditis zealandica* EPNs collected in South Africa. The type strain of the subspecies is SF41<sup>T</sup> (=ATCC BAA-2479<sup>T</sup>=DSM 25263<sup>T</sup>).

**DESCRIPTION OF PHOTORHABDUS HETERORHABDITIS SUBSP. ALUMINESCENS SUBSP. NOV.**

*Photorhabdus heterorhabditis* subsp. *aluminescens* (a.lu. mi. nes′cens. N.L. part. adj. *aluminescens* non-luminescing; for its incapability to produce bioluminescence).

Cells are large motile rods (4.5×1.0–10.0×2.0 µm). Colonies are mucoid, circular, slightly irregular margins, yellow or orange in colour with a diameter of approximately 2 mm after 48 h growth on LB agar. Maximum temperature for growth is 33–34 °C. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and for *H*<sub>2</sub>*S*, indole and acetoin production. Positive for citrate utilization, for urease and gelatinase activity, and for glucose oxidation. Annular haemolysis is observed on sheep blood agar. Natural habitat is *Heterorhabditis* EPNs. The type strain of the subspecies is Q614<sup>T</sup> (=DSM 111144<sup>T</sup>=CCOS 1944<sup>T</sup>=LMG 31959<sup>T</sup>). Whole genome sequences of this strain are available in the NCBI data bank under accession number JABBCS01.
Table 1. API20E-based phenotypic characters to differentiate all *Photorhabdus* species/subspecies. Strains *P. australis* subsp. *thailandensis* subsp. nov. PB68\(^1\), *P. aegyptia* sp. nov. BA1\(^1\), and *P. heterorhabditis* subsp. *aluminescens* subsp. nov. Q614\(^1\) were tested in parallel. The experiment was conducted twice. The obtained results were compared to those published previously [16]. For further information refer to the original studies [9–19,21,23,24,27]. 1: *P. akhurstii*, 2: *P. asymbiotica*, 3: *P. australis* subsp. *australis* subsp. nov., 4: *P. australis* subsp. *thailandensis* subsp. nov., 5: *P. bodei*, 6: *P. caribbeanensis*, 7: *P. cinerea*, 8: *P. aegyptia* sp. nov., 9: *P. hainanensis*, 10: *P. heterorhabditis* subsp. *heterorhabditis* subsp. nov.,\(^{11}\) 11: *P. heterorhabditis* subsp. *aluminescens* subsp. nov.,\(^{12}\) 12: *P. kayaii*, 13: *P. khani* subsp. *khani* subsp. nov.,\(^{14}\) 14: *P. khanii* subsp. *guanajuatensis* subsp. nov.,\(^{15}\) 15: *P. kleinii*, 16: *P. laumondii* subsp. *laumondii*, 17: *P. laumondii* subsp. *clarkei*, 18: *P. luminescens* subsp. *luminescens* subsp. nov., 19: *P. luminiscens* subsp. *mexicana* subsp. nov., 20: *P. namnaonensis*, 21: *P. noenieputensis*, 22: *P. stackebrandtii*, 23: *P. tasmaniensis*, 24: *P. temperata*, 25: *P. thracensis*. “+”: more than 80% of strains and/or conducted tests showed a positive reaction, “-”: more than 80% of strains and/or conducted tests showed a negative reaction, “v”: variable, about 50% of strains/conducted tests showed a positive reaction, “w”: weak, more than 80% of strains and/or conducted tests showed a weak, positive reaction.

| 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   | 21   | 22   | 23   | 24   | 25   |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| β-Galactosidase | –    | –    | w    | w    | –    | –    | –    | –    | –    | v    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | v    |
| Arginine dihydrolase | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | v    | –    | –    | –    | –    | –    | +    | –    |
| Lysine decarboxylase | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Ornithine decarboxylase | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Cit rate utilization | +    | +    | +    | +    | +    | +    | +    | +    | v    | –    | v    | –    | –    | +    | –    | +    | –    | +    | –    | +    | –    | +    | +    | +    | v    |
| H₂S production | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Urease | +    | +    | –    | –    | –    | v    | –    | +    | –    | +    | –    | +    | –    | v    | –    | –    | –    | +    | –    | –    | v    | –    | –    | –    | +    | –    |
| Tryptophan deaminase | –    | –    | v    | v    | +    | +    | –    | –    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | v    | –    | +    | +    | v    |
| Indole production | +    | –    | –    | +    | –    | +    | –    | +    | –    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Acetoin production | +    | –    | –    | –    | +    | –    | +    | –    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Gelatinase | +    | +    | +    | +    | –    | v    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Glucose oxidation | +    | +    | w    | +    | +    | +    | +    | w    | +    | –    | +    | –    | –    | –    | –    | –    | –    | –    | –    | –    | +    | +    | +    | +    | +    |
| Mannitol oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Inositol oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Sorbitol oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Rhamnose oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Sucrose oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Melibiose oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Amygdalin oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| Arabinose oxidation | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| (Cytochrome) oxidase | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| NO₂ production | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| NO₂ reduction to N₂ gas | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | v    |
DESCRIPTION OF PHOTORHABDUS AEGYPTIA SP. NOV.

*Photorhabdus aegyptia* (ae.gyp’t.i.a. L. fam. adj. aegyptia pertaining to Egypt, the country where the entomopathogenic nematodes that host the type strain were originally collected).

Cells are motile, non-spor-forming rods (approx. 1.0 μm wide and 1.5–2.0 μm long). Gram-stain-negative, oxidase-negative and catalase-positive. Colonies are mucoid, circular, slightly irregular margins, pale yellow in colour with a diameter of approximately 2 mm after 48 h growth on LB agar and produce light. Good growth occurs on LB at 28–30°C. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and for H₂S production. Positive for citrate utilization, for urease and gelatinase activity, for glucose oxidation and for indole and acetoin production. The type strain is symbiotically associated with *Heterorhabditis indica* EPNs. Their natural habitat is the intestines of these nematodes and the insects infected by them. The type strain is BA1ᵀ (≡DMS 111180ᵀ=CCOS 1943ᵀ=LMG 31957ᵀ). Whole genome sequences of this strain are available in the NCBI data bank under accession number JFGV01.

**Funding information**  
The work of R.A.R.M. and A.M. is supported by the Swiss National Science Foundation (grant 186 094 to R.A.R.M.). The work of M.A.H. is supported by the National Research Centre (grant 12050137).

**Acknowledgements**  
We thank the Swiss National Science foundation for financial support, the next Generation Sequencing Platform and the Interfaculty Bioinformatics Unit of the University of Bern for performing whole genome sequencing and providing high performance computing infrastructure, the Institute of Biology of the University of Neuchâtel (Switzerland) and the National Research Centre (Cairo, Egypt) for their support.

**Conflicts of interest**  
The authors declare that there are no conflicts of interest.

**References**

5. Lacey LA, Georgis R. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J Nematol 2012;44:218–225.