'*Candidatus Pasteuria aldrichii*', an obligate endoparasite of the bacterivorous nematode *Bursilla*

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A novel bacterium of the genus *Pasteuria* was discovered parasitizing bacterivorous nematodes of the genus *Bursilla*, in selected bermudagrass (*Cynodon*) field plots in Davie, FL, USA. Soil containing this bacterium was sampled and supplied with bi-weekly inoculations of cultured species of the genus *Bursilla* in order to build and maintain a source of endospores for continuous *in vivo* conservation of the bacteria for further study and characterization. 16S rRNA gene sequence similarities supported its congeneric ranking with other members of the genus *Pasteuria* that have been identified from nematodes and cladocerans. There were, however, no clear sister candidates for this organism, which supported the evidence of endospore ultrastructure and host-range studies, suggesting it belonged to a novel taxon. Because members of the genus *Pasteuria* cannot yet be isolated, definitive type strains could not be maintained; therefore, the name '*Candidatus Pasteuria aldrichii*' is proposed for this organism.

The genus *Pasteuria* appears to be a well-defined group of Gram-positive, 'mycelial', endospore-forming, endoparasitic prokaryotes with worldwide distribution, which are found in many different groups of soil-inhabiting nematodes and freshwater cladocerans (Preston *et al.*, 2003). At the time of writing, the genus comprised four recognized species, *Pasteuria ramosa* (Metchnikoff, 1888), *Pasteuria penetrans* (Sayre & Starr, 1985), *Pasteuria thornei* (Starr & Sayre, 1988) and *Pasteuria nishizawae* (Sayre *et al.*, 1991; emended by Noel *et al.*, 2005), and one '*Candidatus*' taxon, '*Candidatus Pasteuria usgae*' (Giblin-Davis *et al.*, 2003).

Although more than 300 members of the genus *Pasteuria* have been reported from many different trophic groups of nematodes, including predatory, free-living, fungivorous and bacterivorous forms, most of the recognized species of the genus *Pasteuria* have been described as being associated

with plant-parasitic nematodes (Chen & Dickson, 1998; Preston *et al.*, 2003; Sturhan *et al.*, 2005). This bias is probably due to a vested interest in obtaining a greater understanding of the potential of these bacteria as biological control agents for this subset of economically important nematodes and is not an indication that any particular affinity of these bacteria for a specific trophic group of nematodes exists (Sturhan *et al.*, 2005).

Because of the obligately parasitic nature of members of the genus *Pasteuria*, culturing has not been possible until recently (Hewlett *et al.*, 2004), and even then is only possible via proprietary means without any suitable contingency for the establishment and sustained maintenance of a standard type culture collection. Previous work, therefore, was done with a typological approach that relied mostly upon the ultrastructure of mature endospores and on the results of host-range studies to delineate recognized taxa (Metchnikoff, 1888; Sayre & Starr, 1985; Sayre *et al.*, 1988, 1991; Starr & Sayre, 1988; Ebert *et al.*, 1996; Giblin-Davis *et al.*, 2001; Atibalentja *et al.*, 2004; Noel *et al.*, 2005). However, advances in sequencing have given rise to a molecular phylogenetic approach for the delineation of

Abbreviations: MCL, maximum composite likelihood; MP, maximum parsimony; NJ, neighbour-joining; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of '*Candidatus Pasteuria aldrichii*' is GU186440.

taxa or phylotypes of members of the genus *Pasteuria* (Ebert *et al.*, 1996; Anderson *et al.*, 1999; Atibalentja *et al.*, 2000; Bekal *et al.*, 2001; Preston *et al.*, 2003; Sturhan *et al.*, 2005; Bishop *et al.*, 2007).

A *Pasteuria*-like bacterium was found parasitizing a bacterivorous nematode of the genus *Bursilla* in soil from bermudagrass (*Cynodon*) field plots at the Fort Lauderdale Research and Education Center, University of Florida, Davie, FL, USA (Giblin-Davis *et al.*, 2004). In an effort to further study and characterize this bacterium, the host was isolated and cultured on TSB (Tryptic Soy Broth) agar. The identity of the host was determined morphologically and molecularly using 18S rDNA, D2D3 LSU rDNA and *mtCOI* gene sequences following the methods of Ye *et al.* (2007). The nematode host was confirmed to have an identical near full-length 18S rDNA gene sequence to *Bursilla* sp. PS1179 (GenBank accession no. U73452) (referred to as *Mesorhabditis* by Baldwin *et al.*, 1997 but as *Bursilla* by Kiontke & Fitch, 2005), which belongs to the *Pleiorhabditis* group of nematode worms Kiontke & Fitch (2005). The host nematode, designated *Bursilla* sp. RGD244, is available from the Fort Lauderdale Research and Education Center and *Bursilla* sp. PS1179 is available from the laboratories of Dr David Fitch and Dr Lynn Carta (<http://www.nyu.edu/projects/fitch/WSRN/strains/ps1179.html>).

In vivo culturing of nematodes/bacteria

Bursilla sp. RGD244 nematodes were mass-produced on mixed bacterial cultures to produce sentry nematodes for identifying soil containing the novel *Pasteuria*-like organism. Once soil from transect sampling was identified as yielding heavily parasitized hosts, it was heated to 50 °C for 2 hours to decontaminate it of other nematodes and supplied with bi-weekly inoculations of cultured populations (~30 000–50 000) of un-infested *Bursilla* sp. RGD244 nematodes in order to build a source of endospores. For initial establishment of *in vivo* culture, un-infested *Bursilla* sp. were inoculated into soil that had been harvested from a single transect location (the original culture was established on 16 July 2003) and their numbers were increased by bi-weekly inoculations of *Bursilla* sp. RGD244 for several months. To reduce potential contamination from other strains of the genus *Pasteuria*, the infested hosts were extracted from the soil using sugar-flotation-centrifugation (Jenkins, 1964) at 48 h post-inoculation and 500 were hand-picked and inoculated into 100 ml autoclaved Margate fine sand with 4 % organic matter (OM). Bi-weekly additions of ~30 000–50 000 un-infested *Bursilla* sp. RGD244 nematodes were made into this soil for 5 months prior to mixing it, as a starter, into 1 l of autoclaved soil in a screened plastic box (13 × 11 × 18 cm). Boxed soil received bi-weekly inoculations of ~30 000–50 000 healthy uninfected *Bursilla* sp. RGD244 nematodes in about 20 ml deionized water for about 1 year before starting over again (Giblin-Davis *et al.*, 2004). This method of building up and maintaining high densities of *Bursilla–Pasteuria* in soil has proven to be reliable over the past 6 years.

Bioassay of these soils after about 2–3 months revealed that un-infested *Bursilla* sp. RGD244 sentinels consistently yielded >80 % of the nematodes of all stages (except the L1 and egg) with 1 to >100 attached bacterial endospores within 24 h of exposure. Replicate experiments assessing the storability of the *Bursilla–Pasteuria* soil culture suggested that dry storage should not be performed for more than about 9 months without renewal of bi-weekly inoculations of cultured populations (30 000–50 000) of un-infested nematodes in order to rejuvenate/maintain the culture. In addition, when the *Bursilla–Pasteuria* soil culture was kept frozen at –20 °C for 5 days and then bioassayed with sentinel nematodes, spores that had attached to the nematodes were observed detaching when heat was applied to immobilize the nematodes for observation. This suggests that freezing is not an acceptable method for storage of *Bursilla–Pasteuria* soil cultures.

Life-cycle description

Timed developmental studies of the *Bursilla–Pasteuria* interaction using soil cultures at room temperature (24–25 °C) (four repeat experiments; *n*=10 for each experiment) showed that a mean of five spores were attached and ~30 % had penetration tubes within 24 h (Fig. 1a). Within the 24 h period after the initial 24 h of exposure (up to 48 h total) to a soil culture, a mean of five spores were attached, ~50 % had penetration tubes and 21 % of the total attached spores were associated with small pseudocoelic microcolonies that were the diameter of the spore or less (Fig. 1b). Within the 48 h period after the initial 24 h exposure time (72 h total), a mean of five spores were attached and 57 % of the total attached spores were associated with small to medium pseudocoelic microcolonies (ranging in length from the diameter of the spore to about 0.5 times the diameter of the nematode). Within the 72 h period after the initial 24 h exposure time (96 h total), a mean of five spores were attached and 52 % of the total attached spores were associated with medium to large pseudocoelic microcolonies (ranging in length from <0.5 to 2 times the diameter of the nematode) (Fig. 1c); there were also many colony fragments visible in the host nematode pseudocoel. Within the 96 h period after the initial 24 h exposure time (120 h total), a mean of six spores were attached, very few of the attached spores were associated with distinct colonies and the nematodes were filled with vegetative stage and maturing endospores, often forming tetrads of spores (each within their mother cell); penetration tubes under spores often appeared more distinct than in previous time periods. Within the 120 h period after the initial 24 h exposure time (144 h total), a mean of six spores were attached and nematodes varied from being completely filled with spores that were showing engulfment and maturation with parasporal fibre formation to being filled with scattered clumps of spores and vegetative cells. Within the 144 h period after the initial 24 h exposure time (168 h total), a mean of four spores were attached and the majority of sampled nematodes were filled with mature endospores (Fig. 1d).

Fig. 1. Light photomicrographs of *Bursilla* sp. RGD244 nematodes at different times post-infection with endospores of 'Candidatus *Pasteuria aldrichii*' showing an attached spore with penetration tube (arrow) after 24 h of exposure to endospores (a), an attached spore with penetration tube and pseudocoelomic microcolony (arrow) on the anterior end of a *Bursilla* sp. RGD244 hermaphrodite after 48 h of exposure to endospores (b), enlarging pseudocoelomic microcolonies (arrows) after 96 h exposure to endospores (c) and sporulation near the pharynx of a *Bursilla* sp. RGD244 nematode after 168 h exposure to endospores (d). Bars, 4 μ m.

Endospore attachment and host-range studies

Host-range attachment studies of the *Bursilla*–*Pasteuria* interaction were conducted using a soil culture and cultured isolates of phylogenetically diverse nematodes, namely: *Bursilla* sp. RGD244 [belonging to the Pleiorhabditis group of Kiontke & Fitch (2005) and clade V of Blaxter *et al.* (1998)]; *Caenorhabditis elegans* (Eurhabditis, clade V); *Acrostichus rhynchophori* RGD193 (Diplogastridae, clade V); *Koerneria* sp. RGD228 (Diplogastridae, clade V); *Myolaimus* sp. RGD233 (Myolaimidae, clade IV); *Aphelenchus avenae* RGD103 (Aphelenchidae, clade IV); *Ditylenchus* sp. RGD199 (Tylenchidae, clade IV) and *Bursaphelenchus xylophilus* (Aphelenchoididae, clade IV). The *Bursilla*–*Pasteuria* soil culture was assayed with *Bursilla* sp. RGD244 sentinel nematodes first and shown to produce high encumbrance levels (100 % of hermaphrodites with a mean cuticular load of 83 spores per nematode) after a 24 h exposure. One litre of this soil was removed and dried for 6 days at room temperature before dividing into 30 g aliquots, each placed into a 35 ml plastic cup. Cultures of each of the above-mentioned species were harvested by the Baermann funnel method (Southey, 1970) and ~1000 nematodes of each species were concentrated into 6 ml water for inoculation. There were four replicate experiments per species. After 24 h of exposure to the culture, 15 g soil per cup was removed, nematodes were extracted separately using the sugar-flotation-centrifugation method (Jenkins, 1964) and >25 nematodes were examined under a compound microscope for attached spores of the *Pasteuria*-like bacterium. Of the *Bursilla* sp. RGD244 nematodes examined, 99 % were encumbered with between 5 and 72 spores. None of the other test species were encumbered with attached spores, nor were *Bursilla* sp. RGD244 nematodes that had been inoculated into autoclaved soil from a soil culture as a control. A month-long

exposure experiment was conducted with the 15 g soil culture remaining in each of the cups by inoculating them with 500 test nematodes in 3 ml deionized water each week for 4 weeks before harvesting again, 24 h after the final inoculation to see if the host's attachment status had changed. Nematodes were harvested as described above. About 90 % of the examined *Bursilla* sp. RGD244 nematodes were encumbered with between 1 and 76 spores. Again, none of the other test species were encumbered with attached spores. Thus, from these broad tests of host specificity within nematode species of clades IV and V, it appears that the *Bursilla*–*Pasteuria* interaction is relatively host-specific, or at least to the level of the Pleiorhabditis group of nematode worms. However, the specificity of the *Bursilla*–*Pasteuria* interaction needs further verification with more members of this group, such as the closely related gonochoristic species of the Pleiorhabditis group, available from the *Caenorhabditis* Genetics Center (CGC), e.g. *Mesorhabditis longespiculosa* DF5017 or *Teratorhabditis palmarum* DF5019.

Bursilla sp. RGD244 nematodes at all stages of development were inoculated into the *Bursilla*–*Pasteuria* soil culture for 96 h and then extracted, measured and examined for encumbrance levels. There were five, relatively discrete, size classes (8–11 nematodes were measured for each size class) of *Bursilla* sp. RGD244: L1 (240–290 μ m); L2 (300–385 μ m); L3 (525–625 μ m); L4 (675–750 μ m) and hermaphroditic females (675–925 μ m). The percentage encumbrance was 0 % for the L1 size class, 38 % for L2, 60 % for L3 and 100 % for L4 and adult hermaphrodites with 1–11 spores attached.

Morphometrics and ultrastructure

Using light microscopy, the mean endospore diameter in the *Bursilla*–*Pasteuria* culture was 4.3 ± 0.2 μ m (range,

3.7–4.8 µm), the mean central body diameter was 1.5 ± 0.1 µm (range, 1.3–1.8 µm) and the mean core diameter was 0.8 ± 0.1 µm (range, 0.7–0.9 µm) ($n=44$ externally attached spores). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to determine the external and internal ultrastructure of the *Bursilla–Pasteuria* complex, relative to other recognized species of the genus *Pasteuria*. For SEM, *Bursilla* sp. RGD244 nematodes were inoculated into the *Bursilla–Pasteuria* soil culture for 24 h and then extracted. Twenty hermaphrodites with spores attached were hand-picked and transferred into 3% (v/v) glutaraldehyde, post-fixed in 2% osmium tetroxide for 12 h at 22 °C, rinsed in deionized water, dehydrated in a graded ethanol series, critical-point dried with liquid CO₂, mounted on a stub with double sticky tape, sputter-coated with 20 nm of gold-palladium and viewed with a Hitachi S-4000 Field Emission scanning electron microscope at 7 kV (Giblin-Davis *et al.*, 2001). For TEM, *Bursilla* sp. RGD244 nematodes were inoculated into the *Bursilla–Pasteuria* soil culture for 24 h and then extracted. Forty hermaphrodites with spores attached were hand-picked and transferred into sterile deionized water and kept at room temperature for 48, 72 and 120 h before being transferred, live, into 2% formaldehyde (prepared from paraformaldehyde) and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and fixed overnight at 4 °C. After repeated rinsing in buffer, specimens were post-fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2 for 3.5 h at 22 °C. The nematodes were rinsed in water, fixed with 1% aqueous uranyl acetate, dehydrated using 100% ethanol, transferred into 100% acetone and infiltrated with Spurr's epoxy resin. Blocks were sectioned on an RMC MT-6000-XL ultra-microtome and nearly-serial sections with silver refraction (75 nm) were picked up on copper grids with a 0.35% Formvar coating reinforced with a light carbon film. Sections were post-stained with 5% aqueous uranyl acetate and lead citrate before viewing in a Zeiss EM-10CA transmission electron microscope at 80 kV. The external and internal ultrastructure of the *Bursilla–Pasteuria* complex was typologically different from all previously described interactions between members of the genus *Pasteuria* and nematode worms (see taxon description) (Fig. 2).

Molecular phylogenetic analysis

For molecular phylogenetic analysis *Bursilla* sp. RGD244 nematodes were inoculated into the *Bursilla–Pasteuria* soil culture for 24 h and then extracted. Twenty hermaphrodites with spores attached were hand-picked, transferred into sterile deionized water and kept at room temperature for 48 h before being transferred into a 0.5 ml tube to be gently smashed with a pipette tip to release the vegetative bacterial cells. The suspension was centrifuged at 5000 g for 5 min and all but 1 µl of the supernatant was removed. The cell pellet was resuspended in 9 µl sample buffer from a GenomiPhi kit (GE healthcare) and heated for 3 min in a

boiling water bath. After cooling on ice for 3 min, 9 µl reaction buffer and 1 µl enzyme mix were added for PCR amplification according to the protocols of the GenomiPhi kit. The 16S rRNA gene fragments were amplified with a general primer pair used for most bacteria (27f: 5'-AGAGTTTGATCMTGGCTCAG-3'; 1100R: 5'-GGGTTG-CGCTCGTTG-3') in a Bio-Rad iCycler using enzyme blends (5 U *Taq* DNA polymerase per reaction and 0.2 U ProofStart DNA polymerase per reaction; Qiagen) and a touchdown PCR protocol with the following conditions: 1 cycle of 95 °C for 5 min, 21 cycles of 95 °C for 30 s followed by an initial annealing temperature of 60 °C for 30 s decreasing by 0.5 °C per cycle and an extension step of 72 °C for 1 min, followed by an additional 20 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final extension step of 72 °C for 10 min. PCR products were obtained at the predicted size of ~1 kb. PCR products were re-treated with *Taq* DNA polymerase (Qiagen) at 72 °C for 10 min and then cloned into pCR4-TOPO following the protocol of the supplier (Invitrogen). The products of ligation were transformed into chemically competent *Escherichia coli* TOP10 cells. Colonies containing recombinant plasmids were chosen for sequencing. Endonuclease restriction digestion demonstrated that the inserts were without an *EcoRI* site. Two sequences of 982 and 1003 bp were obtained from the clones, which showed 99.6% similarity with only four base pairs not matching (three substitutions; one T/C, one T/G and one A/G; and one gap resulting from insertion or deletion).

In order to extend the above-mentioned ~1 kb 16S rRNA gene sequence of the novel *Pasteuria*-like bacteria, bacterial genomic DNA was released from 20 infected nematodes and randomly amplified with a GenomiPhi kit as described above. PCR fragments were amplified with a specific primer for the 5' end of the previously sequenced ~1 kb fragment and a reverse primer containing the consensus sequence (1392r: 5'-ACGGGCGGTGTGTRC-3') at the 3'-end of the gene sequence, using HotStar High Fidelity kit (Qiagen). PCR products were cloned into the pCR2.1-TOPO vector and transformed into *E. coli* TOP10 recipients. Transformants were detected by real-time PCR using an iCycler (Bio-Rad) and determining a product melting point of 89 °C. Seven clones were selected on the basis of RT-PCR parameters (melting point) and the same size of insert being released upon digestion with *EcoRI*. The DNA inserts in these clones were then sequenced. Three of these contained identical sequences for the 540 bp sequence extending from position 853 from the start of the full-length 16S rRNA gene, providing a nearly complete (1393 bp) sequence for the gene encoding 16S rRNA in the novel *Pasteuria*-like bacterium. The four other clones were not related to members of the genus *Pasteuria*, but were most closely related to other members of the phylum *Firmicutes*, for example, members of the families *Paenibacillaceae*, *Enterobacteraceae* and *Xanthomonadaceae* such as species of the genera *Cohnella*, *Photorhabdus* and *Stenotrophomonas*. The complete 16S rRNA sequence

Fig. 2. TEM (a–d) and SEM (e–f) photomicrographs of 'Candidatus Pasteuria aldrichii' from *Bursilla* sp. RGD244. (a) Attached endospore with penetration tube external to cuticular surface of the nematode. (b) Attached and germinating spore that has penetrated the cuticle of the nematode with mycelial stages (arrows) present above and below muscles in the pseudocoelom. (c) Branching and septate (arrows) vegetative stages of 'Candidatus Pasteuria aldrichii' in the pseudocoelom of a *Bursilla* sp. RGD244 nematode. (d) Sporulation of 'Candidatus Pasteuria aldrichii' in the pseudocoelom of a *Bursilla* sp. RGD244 nematode. (e) Attached endospores of 'Candidatus Pasteuria aldrichii' on the cuticle of a *Bursilla* sp. RGD244 nematode. (f) Close-up of attached endospore of 'Candidatus Pasteuria aldrichii' on cuticle of a *Bursilla* sp. RGD244 nematode. Bars; (a–d), 1 μ m; (e), 2.31 μ m; (f), 1.20 μ m. AL, Adhesion layer; C, cortex; N, nucleoid; OSC, outer spore coat; PF, parasporal fibres; PP, penetration peg.

obtained in this study has been made available in GenBank under accession no. GU186440.

For phylogenetic analysis, alignments were performed with sequences of related taxa obtained from GenBank, using CLUSTAL_X version 1.81 using *Mycobacterium tuberculosis* as an outgroup. The aligned results were converted to MEGA version 4.0 (Kumar *et al.*, 2008) format and phylogenetic analyses were performed using MEGA version 4.0 software with the neighbour-joining (NJ), maximum-parsimony (MP) and maximum composite likelihood (MCL) methods (with bootstrap analysis based on 1000 replicates) as described in the manual for the program (<http://www.megasoftware.net/manual.pdf>).

NJ and MP analyses of near full-length 16S rRNA gene sequences (Figs 3 and 4) suggested that the *Pasteuria*-like bacterium occupied a unique taxon, but grouped with other species of the genus *Pasteuria*. Using phylogenetic analyses of multiple loci, Charles *et al.* (2005) inferred that members of the genus *Pasteuria* occur within the Gram-positive, spore-forming *Bacillus*–*Clostridium* supergroup. Therefore, the results of 16S rRNA gene sequence analysis of the *Pasteuria*-like bacterium strongly support the

assertion that it is a previously undocumented organism of the genus *Pasteuria*.

Based on the development, life cycle, morphology, ultra-structure and phylogenetic position of the novel organism, previously referred to as the *Pasteuria* sp. R-1 isolate (Giblin-Davis *et al.*, 2004), it represents a novel taxon in the genus *Pasteuria*, for which the name 'Candidatus Pasteuria aldrichii' is proposed. The bacterium was identified from a bacterivorous nematode of the genus *Bursilla* collected from bermudagrass (*Cynodon*) field-plot soil at the Fort Lauderdale Research and Education Center, University of Florida, FL, USA (26° 05' 12" N 80° 14' 26" W). The following description is based upon data presented herein.

Description of 'Candidatus Pasteuria aldrichii'

'Candidatus Pasteuria aldrichii' (al.dri'chi.i. N.L. masc. gen. n. *aldrichii* of Aldrich, named in honour of Dr Henry Aldrich for his many contributions to research and teaching in microbiology at the University of Florida).

'Candidatus Pasteuria aldrichii' [(Firmicutes) NC; G +; M; NAS (GenBank no. GU186440); morphology (see following

Fig. 3. Molecular analysis of the near full-length 16S rRNA gene sequences of '*Candidatus Pasteuria aldrichii*' and related taxa. The tree was reconstructed with MEGA version 4 software by using the neighbour-joining (NJ) method. Bootstrap values >50 %, based on 1000 replicates, are given at branch points. GenBank accession numbers are given in parentheses. Strain numbers and type strains have been provided where appropriate. Bar, 0.02 substitutions per nucleotide position.

Fig. 4. Molecular analysis of sequences of the near full-length 16S rRNA gene of '*Candidatus Pasteuria aldrichii*'. The tree was reconstructed with MEGA version 4 software by using the maximum-parsimony (MP) method. Bootstrap values >50 %, based on 1000 replicates, are given at branch points. GenBank accession numbers are given in parentheses. Strain numbers and type strains have been provided where appropriate.

description); S (*Bursilla* sp. RGD244 (sp. PS1179), pseudocoelom)] Giblin-Davis *et al.*, 2004, this study.

Obligate endoparasitic bacterium of the pseudocoelom of *Bursilla* nematodes that cannot be cultivated on cell-free media, only by attachment of endospores to species of the genus *Bursilla* and co-cultivation in soil with bi-weekly inoculations of nematodes. Transmission occurs horizontally. Host infection is via cuticular penetration by attached endospores, occurring at all stages of host development, except L1 and egg stages. Sporogenesis, which leads to the death of the host, occurs in the pseudocoelom of L3-stage through to adult-stage nematodes. Sporogenesis is typical of other nematode-specific species of the genus *Pasteuria*. Host range appears to be limited to species of the genus *Bursilla*. Cells are non-motile with a Gram-reaction-positive vegetative phase. 'Mycelium' is septate, hyphal strands branch dichotomously with expansion of hyphal tip forming early sporangium. SEM and TEM reveal dorsal peripheral fibres of the host-attached mature endospore, which are covered with a dense layer of adhesion fibres, except for a slight gap encircling the endospore that appears to be diagnostic for this species. Lateral views with TEM show that the peripheral fibres comprise thin 'wing-like' structures that are narrowly (1/6–1/3 the height of the lateral wall) attached to the anterior-most region of the lateral walls of the spore. This is a diagnostic feature, relative to other species of the genus *Pasteuria* described from nematodes where the peripheral fibres are broadly attached to the lateral walls of the spore (>2/3 the height of the lateral wall). In lateral views with TEM, the ratio of the combined widths of the dorsal and ventral adhesion layers relative to the peripheral fibre layers is about 2:1 in 'Candidatus *Pasteuria aldrichii*' versus ~1:5 in all other described species of bacteria from nematodes. The sporangium and central-body diameters overlap with other described species of the genus *Pasteuria* identified from nematode hosts. In lateral views with TEM, the shape of the central body is a rounded-ellipse, similar to the circular shape seen in *P. ramosa*, which contrasts with the rectangular to rounded-trapezoidal shape seen in transverse sections of 'Candidatus *Pasteuria usgae*', the horizontally oriented elliptical shapes seen in *P. penetrans* and *P. nishizawae*, and the rounded-square shape seen in *P. thornei*. The outer spore coat of 'Candidatus *Pasteuria aldrichii*' is thickest dorsally, and thinnest across the bottom of the spore, which contrasts with 'Candidatus *Pasteuria usgae*', which is seven to eight times thicker laterally than across the bottom, and with all other described species, having outer spore coats with relatively uniform thickness. No basal ring exists around the pore opening, as in *P. penetrans*. There are no lateral microprojections of the outer spore coat as there are in 'Candidatus *Pasteuria usgae*', *P. penetrans* and *P. nishizawae*. The epicortical wall remnant of the mature endospore is difficult to discern in TEM sections but appears to be concentric in lateral view, similar to what has been described for *P. ramosa*, *P. nishizawae* and *P. penetrans*. Spores are not found in distinctive clumps

from the disintegration of the mother thallus as described for the putative 'Candidatus *Pasteuria hartismeri*' (Bishop *et al.*, 2007).

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