‘Candidatus Pasteuria usgae’ sp. nov., an obligate endoparasite of the phytoparasitic nematode Belonolaimus longicaudatus

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Taxonomically relevant characteristics of a fastidiously Gram-positive, obligately endoparasitic prokaryote (strain S-1) that uses the phytoparasitic sting nematode Belonolaimus longicaudatus as its host are reviewed. 16S rDNA sequence similarity (≥93 %) confirms its congeneric ranking with other Pasteuria species and strains from nematodes and cladocerans and corroborates morphological, morphometric and host range evidence suggesting a novel taxon. The 16S rDNA sequence of strain S-1 has greatest similarity (96 %) to the 16S rDNA sequences of both Pasteuria penetrans from root-knot nematodes (Meloidogyne species) and the recently reported strain of Pasteuria isolated from the soybean cyst nematode Heterodera glycines. Because the obligately endoparasitic nature of prokaryotes in the genus Pasteuria prevents isolation of definitive type strains, strain S-1 is proposed as ‘Candidatus Pasteuria usgae’ sp. nov.

There are four nominal species of Pasteuria that are Gram-positive, mycelial, endospore-forming and endoparasitic on nematodes and crustaceans. Pasteuria ramosa (type species) has been described from water fleas (Cladocera: Daphnidae) (Ebert et al., 1996; Metchnikoff, 1888; Sayre et al., 1979, 1983). The other three species are associated with phytoparasitic nematodes. Pasteuria penetrans has been described from root-knot nematodes (Meloidogyne spp.) (Sayre & Starr, 1985; Starr & Sayre, 1988), Pasteuria thornei has been reported from root-lesion nematodes (Pratylenchus spp.) (Starr & Sayre, 1988; Sayre et al., 1988) and Pasteuria nishizawai was found parasitizing cyst nematodes of the genera Heterodera and Globodera (Sayre et al., 1991). Pasteuria strains have been reported attached to and parasitizing numerous nematode species (>300) from around the world (Atibalentja et al., 2000; Ciancio et al., 1994). They are difficult to study because of their highly host-specific and obligately endoparasitic nature (Chen & Dickson, 1998; Dickson et al., 1994, 1996; Giblin-Davis, 2000; Giblin-Davis et al., 1990), which has prevented successful culture in vitro (Bishop & Ellar, 1991; Riese et al., 1988; Williams et al., 1989). Traditional procedures for biochemical characterization are not available for elucidation of Pasteuria species (Sayre & Starr, 1985). The four nominal species of Pasteuria were described using the Linnaean species concept based upon discontinuities in morphometrics, ultrastructure of mature endospores and host attachment specificity. The ultrastructure of the mature endospore of strain S-1, a Gram-positive, obligately endoparasitic prokaryote that uses the phytoparasitic sting nematode Belonolaimus longicaudatus as its host, is distinctive when compared with endospores of named species of Pasteuria (Giblin-Davis, 2000; Giblin-Davis et al., 1990, 1995, 2001). The terminology used herein to describe endospore morphology was previously defined by Sturhan et al. (1994).

P. ramosa Metchnikoff 1888 was described from the water fleas Daphnia magna and Daphnia pulex. All attempts at culture failed and a type strain was not established.
(Metchnikoff, 1888). Years of confusion ensued, as the original classification of *P. ramosa* as a bacterium was challenged with suggestions that it was a *Torula* yeast, a microsporidian or a member of the haplosporidian genera *Democystidium* or *Lymphocystidium* (Ebert et al., 1996). The situation was complicated further when Hirsch (1972) and Staley (1973) used the name *P. ramosa* Metchnikoff 1888 for a superficially similar budding bacterial species found on the exterior surfaces of *Daphnia* species. This prokaryote (strain ATCC 27377) was cultivated *in vitro* and erroneously assigned as the type species of the genus *Pasteuria*, even though it did not form endospores, mycelia or branches, was not an endoparasite of cladocerans and had a Gram-negative reaction (Staley, 1973). Strain ATCC 27377 was subsequently reclassified as *Planctomyces staleyi* Starr, Sayre & Schmidt 1983 (Starr et al., 1983). The latter authors requested conservation of the original description of *P. ramosa* Metchnikoff 1888 and rejection of ATCC 27377 as the type strain. That request was supported by the Judicial Commission of the International Committee for Systematic Bacteriology (1986). Strain ATCC 27377 was then assigned to *Pirella staleyi* by Schlesner & Hirsch (1984), which did not have priority because a fungal genus already occupied the name *Pirella*. Thus, a new genus, *Pirellula*, was created with strain ATCC 27377 being named *Pirellula staleyi* (Schlesner & Hirsch 1984) by Schlesner & Hirsch (1987).

A *P. ramosa*-like strain was discovered infecting *Moina rectirostris*, a member of the Daphnidae (Sayre et al., 1977), and this strain was used in the emendation of the species (Starr et al., 1983). However, Ebert et al. (1996) have proposed that the *Daphnia*-parasitic *P. ramosa* they characterized from the same host as Metchnikoff (1888) be designated as the neotype for *P. ramosa* Metchnikoff 1888 and that the *Moina* isolate be compared directly to the neotype in future studies.

Because the obligately endoparasitic nature of *Pasteuria* currently prevents isolation of a definitive type strain, ‘*Candidatus*’ status is proposed for each novel provisional species designation in this genus (see Murray & Schleifer, 1994; Murray & Stackebrandt, 1995; Stackebrandt et al., 2002). All of the currently named species in the genus *Pasteuria* Metchnikoff 1888 have nomenclatural standing and remain validly named species. These species are *P. ramosa* Metchnikoff 1888 (Approved Lists 1980) emend. Starr et al. 1986 [with the description of Metchnikoff (1888) as emended by Starr et al. (1983) serving as the type: see Judicial Commission of the International Committee on Systematic Bacteriology (1986); Wayne (1986)]. *P. nishizawai* Sayre et al. 1992 [description and illustrations from Sayre et al. (1991) serving as type], *P. penetrans* (ex Thorne 1940) Sayre & Starr 1986 [description and illustrations from Sayre & Starr (1985) serving as type] and *P. thornei* Starr & Sayre 1988 [description and illustrations from Sayre et al. (1988) serving as type]. We concur with the proposal by Ebert et al. (1996) to accept the *Daphnia* parasite that they isolated, studied and sequenced as the neotype for *P. ramosa* and the genus *Pasteuria*. Unfortunately, 16S rDNA sequence data are not available for *P. thornei* or *P. nishizawai* and these forms must be rediscovered before a more complete characterization of these *Pasteuria* and ‘*Candidatus Pasteuria*’ species can be made.

Recent phylogenetic analyses of 16S rDNA sequences from *P. ramosa* (U34688) (Ebert et al., 1996), *P. penetrans* (AF077672) (Anderson et al., 1999; Bekal et al., 2001), ‘*Candidatus Pasteuria* sp.’ strain HG ex *Heterodera glycines* (AF134868) (Atibalentja et al., 2000) and ‘*Candidatus Pasteuria* sp.’ strain S-1 ex *B. longicaudatus* (AF254387) (Bekal et al., 2001) have shown that *Pasteuria* is embedded in the same clade as members of the genus *Alicyclobacillus* (Atibalentja et al., 2000; Ebert et al., 1996). However, there is some support for *Pasteuria* as a separate clade originating between the *Alicyclobacillus* and *Thermoactinomyces* clades (Atibalentja et al., 2000). In addition, *Pasteuria* shared greatest sequence identity with *Thermoactinomyces* (about 87%) (Atibalentja et al., 2000). 16S rDNA sequence analysis corroborates ultrastructural and host attachment studies that support a novel species designation for a ‘*Candidatus Pasteuria*’ collected from the sting nematode *B. longicaudatus* (Bekal et al., 2001; Giblin-Davis, 2000; Giblin-Davis et al., 2001). The 16S rDNA sequence corresponding to nucleotide positions 28–1390 of strain S-1 had similarity to previously published sequences of 96% to *P. penetrans* (Anderson et al., 1999), 93% to *P. ramosa* (Ebert et al., 1996) and 96% to ‘*Candidatus Pasteuria* sp.’ strain HG of *H. glycines* (Atibalentja et al., 2000). Bootstrap analysis using maximum-likelihood, maximum-parsimony and minimum evolution showed strong support for a phytoparasitic nematode-associated clade (100%) that excluded *P. ramosa* (Bekal et al., 2001). The two geographical strains of *P. penetrans* (Senegal and Florida) formed a robust clade (88–100%), whereas strain S-1 was part of a weakly supported clade together with ‘*Candidatus Pasteuria* sp.’

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**Fig. 1.** Maximum-likelihood phylogram for the 16S rRNA genes (1389 bp) of *P. penetrans* strains P100—P20 (Anderson et al., 1999) and Pp (Bekal et al., 2001), *P. ramosa* (Ebert et al., 1996), ‘*Candidatus Pasteuria usgae*’ sp. nov. (Bekal et al., 2001) and ‘*Candidatus Pasteuria* sp.’ strain HG ex *H. glycines* (Atibalentja et al., 2000). *Thermoactinomyces peptonophilus* was used as the outgroup. Bootstrap support percentages for clades A, B and C are respectively 100, 100 and 54%. Bar: 0·01 substitutions per site.
strain HG of H. glycines (41–64%) (Bekal et al., 2001). These data support the hypothesis that S-1 represents a novel species of Candidatus Pasteuria’ (Bekal et al., 2001; Giblin-Davis, 2000; Giblin-Davis et al., 1990, 1995, 2001).

The name ‘Candidatus Pasteuria usgae’ sp. nov. is proposed for the Candidatus Pasteuria sp. from the sting nematode B. longicaudatus from southern Florida [Fort Lauderdale Research and Education Center, Davie, Florida (26°05’N, 080°14’26”W)], previously referred to as an isolate of the P. penetra group (Giblin-Davis, 1991; Giblin-Davis et al., 1990), a large-spored isolate of Pasteuria sp. from B. longicaudatus (Dickson et al., 1994), or as various designations involving Pasteuria and S-1 (Bekal et al., 1999, 2001; Brito et al., 2000; Giblin-Davis, 2000; Giblin-Davis et al., 1995, 1998, 2001). The description is based upon data and comparisons of S-1 with other described species of Pasteuria in three recently published papers (Bekal et al., 2001; Giblin-Davis, 2000; Giblin-Davis et al., 2001).

Description of ‘Candidatus Pasteuria usgae’ sp. nov.

‘Candidatus Pasteuria usgae’ (u.s.g’a.e. N.L. gen. n. usgae of USGA, the acronym for the United States Golf Association, in gratitude for their financial support to study this potential biological control agent against B. longicaudatus in turfgrass ecosystems).

‘Candidatus Pasteuria usgae’ ([Firmicutes] NC; G+; M; NAS (GenBank no. AF254387), morphology (see following description); S (Belonolaimus longicaudatus, pseudocoelom)). Obligate endoparasitic bacterium of the pseudocoelom of B. longicaudatus that cannot be cultivated on cell-free media, only by attachment of endospores to B. longicaudatus and co-cultivation on excised axenic root or greenhouse plant cultures. Transmission occurs horizontally. Host infection is via cuticular penetration by attached endospores that occurs on all stages of B. longicaudatus except eggs. Sporogenesis, which leads to the death of the host, occurs in the pseudocoelom of J3 through adult stage nematodes. Sporogenesis is typical of other nematode-specific Pasteuria. Host range appears to be limited to B. longicaudatus, although attachment of endospores has been observed on Belonolaimus euthychilus, but not on other soil-inhabiting nematodes. Organism is non-motile with Gram-positive vegetative phase. Mycelium is separte, hyphal strands branch dichotomously with expansion of hyphal tip forming sporangium. SEM observation shows that peripheral fibres of the mature endospore protrude around the exposed spherical outer coat of the spore creating a crenate border, as opposed to the other species of Pasteuria described from nematodes, which have no scalloped border. The sporangium and central body diameters are on average at least 0.7 and 0.5 µm wider than these respective measurements for the other described species of Pasteuria. In lateral view with TEM, the shape of the central body of S-1 is a rounded rectangle to a rounded trapezoid in transverse section, which contrasts with the circular shape of P. ramosa, the horizontally oriented elliptical shapes of P. penetra and P. nishizawai and the rounded-square shape of P. thornei. The outer spore coat is thickest laterally, thinner on top and thinnest across the bottom of the spore, being 7–8 times thicker laterally than along the bottom. These measurements contrast with all other described species, having outer spore coats with relatively uniform thickness. No basal ring exists in S-1 around the pore opening as in P. penetra. The outer coat wall thickness at its thickest point is >15% (both walls >30%) of the diameter of the central body, compared with 3 to <13% (both walls 6 to <25%) for the other described species of Pasteuria. The epicotylar wall remnant of the mature endospore occurs between the cortex and the inner spore coat in a sublateral band, similar to P. thornei, but different from the other three described species. The epicotylar walls in the other described species are as follows: completely concentric in P. ramosa and P. nishizawai and lateral in P. penetra.

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