International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 197–200 **DOI** 10.1099/ijs.0.02292-0

Note

‘*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 ($\geqslant$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 nishizawae* 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; Ebert *et al.*, 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

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The GenBank accession number for the 16S rDNA sequence of strain S-1 is AF254387.

(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.

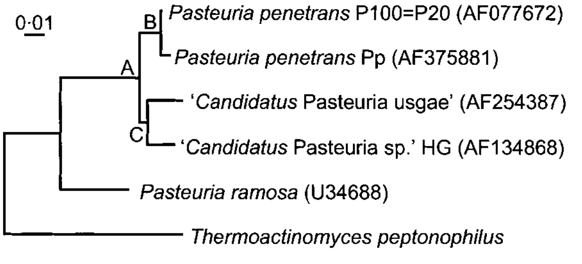

Fig. 1. Maximum-likelihood phylogram for the 16S rRNA genes (1389 bp) of *P. penetrans* strains P100=P20 (Anderson *et al.*, 1999) and Pp (Bekal *et al.*, 2001), *P. ramosa* (Ebert *et al.*, 1996), '*Candidatus* Pasteuria usgae' sp. nov. (Bekal *et al.*, 2001) and '*Candidatus* Pasteuria sp.' strain HG ex *Heterodera 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.

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. nishizawae* 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. nishizawae* 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.'

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′12″N, 080°14′26″W)], previously referred to as an isolate of the *P. penetrans* 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 septate, 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. penetrans* and *P. nishizawae* 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. penetrans*. 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 epicortical 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 epicortical walls in the other described species are as follows: completely concentric in *P. ramosa* and *P. nishizawae* and lateral in *P. penetrans*.

Acknowledgements

Special thanks to Henry Aldrich, Barbara J. Center and Z. X. Chen for help and discussions concerning this project and to Monica Elliott and Nigel Harrison for reviewing the manuscript. This research was supported by a grant from the United States Golf Association. This manuscript is Florida Agricultural Experiment Stations Journal Series R-08596.

References

Anderson, J. M., Preston, J. F., Dickson, D. W., Hewlett, T. E., Williams, N. H. & Maruniak, J. E. (1999). Phylogenetic analysis of *Pasteuria penetrans* by 16S rRNA gene cloning and sequencing. *J Nematol* **31**, 319–325.

Atibalentja, N., Noel, G. R. & Domier, L. L. (2000). Phylogenetic position of the North American isolate of *Pasteuria* that parasitizes the soybean cyst nematode, *Heterodera glycines*, as inferred from the 16S rDNA sequence analysis. *Int J Syst Evol Microbiol* **50**, 605–613.

Bekal, S., Giblin-Davis, R. M. & Becker, J. O. (1999). Gnotobiotic culture of *Pasteuria* sp. on *Belonolaimus longicaudatus*. *J Nematol* **31**, 522.

Bekal, S., Borneman, J., Springer, M. S., Giblin-Davis, R. M. & Becker, J. O. (2001). Phenotypic and molecular analysis of a *Pasteuria* strain parasitic to the sting nematode. *J Nematol* **33**, 110–115.

Bishop, A. H. & Ellar, D. J. (1991). Attempts to culture *Pasteuria penetrans* in vitro. *Biocontrol Sci Technol* **1**, 101–114.

Brito, J. A., Preston, J. F., Dickson, D. W., Giblin-Davis, R. M. & Aldrich, H. (2000). Immunolocalization of adhesins in *Pasteuria* spp. *J Nematol* **32**, 421.

Chen, Z. X. & Dickson, D. W. (1998). Review of *Pasteuria penetrans*: biology, ecology, and biological control potential. *J Nematol* **30**, 313–340.

Ciancio, A., Bonsignore, R., Vovlas, N. & Lamberti, F. (1994). Host records and spore morphometrics of *Pasteuria penetrans* group parasites of nematodes. *J Invertebr Pathol* **63**, 260–267.

Dickson, D. W., Oostendorp, M., Giblin-Davis, R. M. & Mitchell, D. J. (1994). Control of plant-parasitic nematodes by biological antagonists. In *Pest Management in the Subtropics. Biological Control – A Florida Perspective*, pp. 575–601. Edited by D. Rosen, F. D. Bennett & J. L. Capinera. Andover, UK: Intercept.

Ebert, D., Rainey, P., Embley, T. M. & Scholz, D. (1996). Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philos Trans R Soc Lond B* **351**, 1689–1701.

Giblin-Davis, R. M. (1991). Potential for biological control of phytoparasitic nematodes in bermudagrass turf with isolates of the *Pasteuria penetrans* group. *Proc Annu Meet Fla State Hortic Soc* **103**, 349–351.

Giblin-Davis, R. M. (2000). *Pasteuria* sp. for biological control of the sting nematode, *Belonolaimus longicaudatus*, in turfgrass. In *Fate and Management of Turfgrass Chemicals*, American Chemical Society Symposium Series no. 743, pp. 408–426. Edited by J. M. Clark & M. P. Kenna. New York: Oxford University Press.

Giblin-Davis, R. M., McDaniel, L. L. & Bilz, F. G. (1990). Isolates of the *Pasteuria penetrans* group from phytoparasitic nematodes in bermudagrass turf. *J Nematol* **22** (Suppl. 4), 750–762.

Giblin-Davis, R. M., Williams, D., Hewlett, T. E. & Dickson, D. W. (1995). Development and host attachment studies using *Pasteuria* from *Belonolaimus longicaudatus* from Florida. *J Nematol* **27**, 500.

Giblin-Davis, R. M., Center, B. J., Hewlett, T. E. & Dickson, D. W. (1998). *Pasteuria*-infested soil suppresses *Belonolaimus longicaudatus* in a bermudagrass green. *J Nematol* **30**, 497.

Giblin-Davis, R. M., Williams, D. S., Wergin, W. P., Dickson, D. W., Hewlett, T. E., Bekal, S. & Becker, J. O. (2001). Ultrastructure and development of *Pasteuria* sp. (S-1 strain), an obligate endoparasite of *Belonolaimus longicaudatus* (Nemata: Tylenchida). *J Nematol* **33**, 227–238.

Hirsch, P. (1972). Re-evaluation of *Pasteuria ramosa* Metchnikoff 1888, a bacterium pathogenic for *Daphnia* species. *Int J Syst Bacteriol* **22**, 112–116.

Judicial Commission of the International Committee for Systematic Bacteriology (1986). Opinion 61. Rejection of the type strain of *Pasteuria ramosa* (ATCC 27377) and conservation of the species *Pasteuria ramosa* Metchnikoff 1888 on the basis of the type descriptive material. *Int J Syst Bacteriol* **36**, 119.

Metchnikoff, M. E. (1888). *Pasteuria ramosa* un représentant des bactéries à division longitudinale. *Ann Inst Pasteur* **2**, 165–170.

Murray, R. G. E. & Schleifer, K. H. (1994). Taxonomic notes: a proposal for recording the properties of putative taxa of prokaryotes. *Int J Syst Bacteriol* **44**, 174–176.

Murray, R. G. E. & Stackebrandt, E. (1995). Taxonomic note: implementation of the provisional *Candidatus* status for incompletely described prokaryotes. *Int J Syst Bacteriol* **45**, 186–187.

Riese, R. W., Hackett, K. J., Sayre, R. M. & Huettel, R. N. (1988). Factors affecting cultivation of three isolates of *Pasteuria* spp. *J Nematol* **20**, 657.

Sayre, R. M. & Starr, M. P. (1985). *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. *Proc Helminthol Soc Wash* **52**, 149–165.

Sayre, R. M., Wergin, W. P. & Davis, R. E. (1977). Occurrence in *Moina rectirostris* (Cladocera: Daphnidae) of a parasite morphologically similar to *Pasteuria ramosa* (Metchnikoff 1888). *Can J Microbiol* **23**, 1573–1579.

Sayre, R. M., Adams, J. R. & Wergin, W. P. (1979). Bacterial parasite of a cladoceran: morphology, development in vivo, and taxonomic relationships with *Pasteuria ramosa* Metchnikoff 1888. *Int J Syst Bacteriol* **29**, 252–262.

Sayre, R. M., Gherna, R. L. & Wergin, W. P. (1983). Morphological and taxonomic reevaluation of *Pasteuria ramosa* Metchnikoff 1888 and "*Bacillus penetrans*" Mankau 1975. *Int J Syst Bacteriol* **33**, 636–649.

Sayre, R. M., Starr, M. P., Golden, A. M., Wergin, W. P. & Endo, B. Y. (1988). Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore-forming bacterial parasite from *Pratylenchus brachyurus*. *Proc Helminthol Soc Wash* **55**, 28–49.

Sayre, R. M., Wergin, W. P., Schmidt, J. M. & Starr, M. P. (1991). *Pasteuria nishizawae* sp. nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera*. *Res Microbiol* **142**, 551–564.

Schlesner, H. & Hirsch, P. (1984). Assignment of ATCC 27377 to *Pirella* gen. nov. as *Pirella staleyi* comb. nov. *Int J Syst Bacteriol* **34**, 492–495.

Schlesner, H. & Hirsch, P. (1987). Rejection of the genus name *Pirella* for pear-shaped budding bacteria and proposal to create the genus *Pirellula* gen. nov. *Int J Syst Bacteriol* **37**, 441.

Stackebrandt, E., Frederiksen, W., Garrity, G. M. & 10 other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

Staley, J. T. (1973). Budding bacteria of the *Pasteuria–Blastobacter* group. *Can J Microbiol* **19**, 609–614.

Starr, M. P. & Sayre, R. M. (1988). *Pasteuria thornei* sp. nov. and *Pasteuria penetrans* sensu stricto emend., mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne*. *Ann Inst Pasteur Microbiol* **139**, 11–31.

Starr, M. P., Sayre, R. M. & Schmidt, J. M. (1983). Assignment of ATCC 27377 to *Planctomyces staleyi* sp. nov. and conservation of *Pasteuria ramosa* Metchnikoff 1888 on the basis of type descriptive material. Request for an Opinion. *Int J Syst Bacteriol* **33**, 666–671.

Sturhan, D., Winkelheide, R., Sayre, R. M. & Wergin, W. P. (1994). Light and electron microscopical studies of the life cycle and developmental stages of a *Pasteuria* isolate parasitizing the pea cyst nematode, *Heterodera goettingiana*. *Fundam Appl Nematol* **17**, 29–42.

Thorne, G. (1940). *Duboscqia penetrans* n. sp. (Sporozoa: Microsporidia, Nosematidae), a parasite of the nematode *Pratylenchus pratensis* (de Man) Filipjev. *Proc Helminthol Soc Wash* **7**, 51–53.

Wayne, L. G. (1986). Actions of the Judicial Commission of the International Committee on Systematic Bacteriology on requests for opinions published in 1983 and 1984. *Int J Syst Bacteriol* **36**, 357–358.

Williams, A. B., Stirling, G. R., Hayward, A. C. & Perry, J. (1989). Properties and attempted culture of *Pasteuria penetrans*, a bacterial parasite of root-knot nematodes (*Meloidogyne javanica*). *J Appl Bacteriol* **67**, 145–156.