Request for an Opinion

Correspondence

David R. Arahal
arahal@uv.es

The status of the species *Beijerinckia fluminensis* Döbereiner and Ruschel 1958. Request for an Opinion

Monike Oggerin,¹ Víctor Rubio,² Irma Marín³ and David R. Arahal⁴

¹Instituto Nacional de Técnica Aeroespacial, INTA-CSIC, 28850 Torrejón de Ardoz, Madrid, Spain

²Instituto de Ciencias Agrarias, CSIC, 28006 Madrid, Spain

³Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain

⁴Colección Española de Cultivos Tipo (CECT), Parc Científic Universitat de València, 46980 Paterna; and Departamento de Microbiología y Ecología, Universidad de Valencia, 46100 Burjassot, Valencia, Spain

In a previous article [Oggerin M., Arahal, D. R., Rubio, V. & Marín, I. (2009). *Int J Syst Evol Microbiol* **59**, 2323–2328], it has been shown that strain *Beijerinckia fluminensis* UQM 1685^T and its derived equivalent *B. fluminensis* CIP 106281^T do not conform to the description of the type strain of *Beijerinckia fluminensis* Döbereiner and Ruschel 1958. Indeed, both strains were identified as members of the species *Rhizobium radiobacter* and exhibited marked phenotypic and genotypic differences with members of the genus *Beijerinckia*. It was concluded that both strains, and any other equivalents derived from them, do not descend from the nomenclatural type. Since then, our attempts to find older deposits of the type strain, hopefully derived from the original isolate, or other existing strains of *Beijerinckia fluminensis* that could be proposed as a neotype strain, have been in vain. It is therefore proposed that the Judicial Commission should place the name *Beijerinckia fluminensis* Döbereiner and Ruschel 1958 on the list of rejected names if a suitable replacement type strain or a neotype cannot be found within two years following the publication of this Request (Rule 18c).

During the course of a research project with free-living nitrogen fixing bacteria, we confirmed that strains *Beijerinckia fluminensis* UQM 1685^T and *B. fluminensis* CIP 106281^T have the same origin but do not descend from the nomenclatural type since they exhibit important phenotypic and genotypic differences when compared with the descriptions of *B. fluminensis* or any of the other species of the genus *Beijerinckia*. They could be identified as *Rhizobium radiobacter* (Oggerin *et al.*, 2009).

B. fluminensis was first isolated from acidic (pH 4.2–5.2) soil samples collected in locations from four Brazilian states (Döbereiner & Ruschel, 1958). As of February 2011, the StrainInfo.net biportal (Dawyndt *et al.*, 2005) displayed the following culture collection numbers as equivalents of the type strain CD10: CCUG 53676, CIP 106281, DSM 2327, NCAIM B.01797 and UQM 1685. The succession of exchanges and deposits could be reconstructed as follows: UQM 1685 → DSM 2327 → (Varga, Sz.) → NCAIM B.01797 → CIP 106281. Strain DSM 2327 was also delivered to E. R. B. Moore, Aberdeen, UK, who later deposited it at the CCUG in 2006 (<http://www.ccug.se/>). The base of this line (UQM 1685) and one of its tips

(CIP 106281) had already been tested by Oggerin *et al.* (2009) and identified as *R. radiobacter* as mentioned above. At that time we also noticed that strain DSM 2327 was no longer in the DSMZ catalogue (<http://www.dsmz.de/>) and this is still the case as of February 2011. As for strain CCUG 53676, the text displayed in 2008 ('original DSM ampoules, not yet processed') has been replaced by a full and very informative entry that includes a long list of coded phenotypic features. We have examined these features and, as expected, they fit with the characteristics determined for strains UQM 1685 and CIP 106281 (Oggerin *et al.*, 2009). We also obtained strains DSM 2327 and CCUG 53676 from their respective collections and confirmed by means of 16S rRNA gene sequence analysis and the characterization of key phenotypic traits (Oggerin *et al.*, 2009) that, as expected, these strains were indistinguishable from strains UQM 1685 and CIP 106281. In summary, all this evidence proves that strain UQM 1685 and subsequent deposits actually correspond to a strain of *R. radiobacter* (Table 1).

It is impossible to determine without further evidence whether the material that arrived at the UQM culture collection was already the incorrect strain or whether a

Table 1. Differential characteristics between strains designated as *B. fluminensis* and related strains

Taxa: 1, *B. fluminensis* UQM 1685^T and subcultures maintained in other collections; 2, *B. fluminensis* (Döbereiner & Ruschel, 1958; Thompson & Skerman, 1979); 3, *R. radiobacter* CECT 4112^T. +, Positive; –, negative. Further differences between *B. fluminensis* UQM 1685^T and the descriptive data for *B. fluminensis* are given in Oggerin *et al.* (2009).

Characteristic	1	2	3
Aerobic N ₂ fixation	–	+*†	–
Growth at 37 °C	+	–*†	+
Growth on peptone medium	+	–*†	+
Urease	+	–†	–
Assimilation of starch	+	–*	+
Hydrolysis of aesculin	+	–†	+
DNA G + C content (mol%)	60.7	56.2†	61.0

*Döbereiner & Ruschel, (1958).

†Thompson & Skerman, (1979).

human error occurred afterwards. The equivalence between the CD10 and UQM 1685 strain designations could also not be confirmed. This information appears not only in StrainInfo, but also in Becking (1984) and later in Kennedy (2005) with the addition of DSM 2327. In Döbereiner & Ruschel (1958), one of the twelve strains that conform to the first description of the species is designated CD₁₀ (with the number ten as subscript). Although none of the strains was appointed as the nomenclatural type, this situation was not exceptional at that time. Indeed a coetaneous publication that emphasized the importance for modern bacteriological taxonomy of designating type strains also noted that they were lacking for most species of bacteria (Sneath & Cowan, 1958).

In an attempt to obtain any of the original, or even later, isolates of *B. fluminensis*, we contacted the Coleção Bactérias Diazotróficas at Embrapa Agrobiologia (Brazil). Unfortunately, we were informed by its curator that no strains could be recovered from the old stocks (R. M. Pitard, personal communication). Similar direct searches in other collections were also in vain.

Most of the literature related to *B. fluminensis* goes back to the 1960s or before (Amor Asunción *et al.*, 1980; Becking, 1959, 1961, 1974; Döbereiner, 1961; Döbereiner & Ruschel, 1958; Hilger, 1965; Moore, 1963; Thompson & Skerman, 1979) and the only evidence of publicly available strains we could obtain is that displayed in StrainInfo.net (Dawyndt *et al.*, 2005). Strains LMG 2819, NCIMB 9881, NCIMB 9882, NCIMB 11068, NCIMB 11069 have already been characterized by Oggerin *et al.* (2009) who found they represented a distinct species of the genus *Beijerinckia*, *B. doebereineriae* (type strain LMG 2819^T=CECT 7311^T).

Types are of major importance in prokaryote taxonomy (Tindall *et al.*, 2010). In the case of a species or a

subspecies, the designation of type strains is regulated by the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) through Rules 18a–18g and 19. Alterations to this Code have been made by the International Committee on Systematic Bacteriology (ICSB) and the International Committee on Systematics of Prokaryotes (ICSP) and the changes proposed by the Judicial Commission (De Vos & Trüper, 2000) were accepted at one of its plenary sessions. In the following paragraphs the new wording introduced by these recent alterations is used, although the discussion could be also sustained with that of the last printed version (Lapage *et al.*, 1992).

According to Rule 18b, if the author in the effective or valid publication of the name of a species or subspecies definitely designated a type strain, then this strain shall be accepted as the type strain and may be referred to as the holotype (Lapage *et al.*, 1992; De Vos & Trüper, 2000). The valid publication of the name *B. fluminensis* is that of Skerman *et al.* (1980). This species has its entry on pages 262–263 and, after crediting Döbereiner & Ruschel (1958) for the name, strain UQM 1685 is designated as the type (thus, holotype). A book chapter by Thompson & Skerman (1979) is cited as giving the description of the species. The description referred to is the result of a large taxonomic study conducted on free-living aerobic nitrogen-fixing bacteria and is presented in the same book. In the case of *B. fluminensis* it is consistent, but much more detailed, with the description given in Döbereiner & Ruschel (1958). The data were collected from ten strains considered to be members of the species: one of them (strain F1-100=WR-162) was donated by J. Döbereiner who received it from Y. T. Dommergues who isolated it from soil in the Congo. The remaining nine strains were isolated from Australian soils by J. P. Thompson. Another strain of *B. fluminensis* (F1-60 from Brazilian soil) donated as a cotype by J. Döbereiner was considered not typical of the species. The authors of the book chapter concluded that strain WR-162 would be suitable for designation as a neotype strain if authentic cotype strains were no longer extant. The term cotype is not defined in the Bacteriological Code (Lapage *et al.*, 1992) or in its previous printed versions (Buchanan *et al.*, 1948, 1958; The Editorial Board of the Judicial Commission of the International Committee on Nomenclature of Bacteria, 1966), but it is used in various papers dealing with bacterial taxonomy (Buchanan, 1962; Sneath & Cowan, 1958; Sneath & Skerman, 1966) and defined as ‘any specimen of the describing author’s collection if he did not designate a holotype strain’ (Sneath & Cowan, 1958). Unfortunately, to the best of our knowledge all of the strains used in the description by Thompson & Skerman (1979) have been lost.

According to Rule 18c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), if a suitable replacement type strain or a neotype cannot be found or proposed, respectively, within two years of the publication of this Request for an Opinion, it is proposed that the

Judicial Commission places the name *Beijerinckia fluminensis* on the list of rejected names.

Acknowledgements

This work was supported by a grant from Soil Biogenics Ltd. M. O. was funded by a fellowship from Soil Biogenics Ltd.

References

- Amor Asunción, J. M., Frontera, G. & Dellepiane, E. (1980).** [Species of the genus *Beijerinckia* Derx isolated from a soil of Corrientes (Argentina)]. *Rev Argent Microbiol* **12**, 23–28. (in Spanish).
- Becking, J. H. (1959).** Nitrogen-fixing bacteria of the genus *Beijerinckia* in South African soils. *Plant Soil* **11**, 193–206.
- Becking, J. H. (1961).** Studies on nitrogen-fixing bacteria of the genus *Beijerinckia*. I. Geographical and ecological distribution in soils. *Plant Soil* **14**, 49–81.
- Becking, J. H. (1974).** Nitrogen-fixing bacteria of the genus *Beijerinckia*. *Soil Sci* **118**, 196–212.
- Becking, J. H. (1984).** Genus *Beijerinckia*. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 311–321. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Buchanan, R. E. (1962).** Typification in bacteriology. *Int Bull Bacteriol Nomencl Taxon* **12**, 17–26.
- Buchanan, R. E., St John-Brooks, R. & Breed, R. S. (1948).** International bacteriological code of nomenclature. *J Bacteriol* **55**, 287–306.
- Buchanan, R. E., Wikén, T., Cowan, S. T. & Clark, W. A. (1958).** *International Code of Nomenclature of Bacteria and Viruses: Bacteriological Code*. Ames: Iowa State College Press.
- Dawyndt, P., Vancanneyt, M., De Meyer, H. & Swings, J. (2005).** Knowledge accumulation and resolution of data inconsistencies during the integration of microbial information sources. *IEEE Trans Knowl Data Eng* **17**, 1111–1126.
- De Vos, P. & Trüper, H. G. (2000).** Judicial Commission of the International Committee on Systematic Bacteriology. IXth International (IUMS) Congress of Bacteriology and Applied Microbiology. Minutes of the meetings, 14, 15 and 18 August 1999, Sydney, Australia. *Int J Syst Evol Microbiol* **50**, 2239–2244.
- Döbereiner, J. (1961).** Nitrogen-fixing bacteria of the genus *Beijerinckia* Derx in the rhizosphere of sugar cane. *Plant Soil* **15**, 211–216.
- Döbereiner, J. & Ruschel, A. P. (1958).** Uma nova espécie de *Beijerinckia*. *Rev Biol* **1**, 261–272. (in Portuguese).
- Hilger, F. (1965).** [Study on the systematics of the genus *Beijerinckia* Derx]. *Ann Inst Pasteur (Paris)* **109**, 406–423. (in French).
- Kennedy, C. (2005).** Genus I. *Beijerinckia*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2C, pp. 423–432. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992).** *International Code of Nomenclature of Bacteria (1990 Revision)*. Bacteriological Code. Washington, DC: American Society for Microbiology.
- Moore, A. W. (1963).** Occurrence of non-symbiotic nitrogen-fixing micro-organisms in Nigerian soils. *Plant Soil* **19**, 385–395.
- Oggerin, M., Arahall, D. R., Rubio, V. & Marin, I. (2009).** Identification of *Beijerinckia fluminensis* strains CIP 106281^T and UQM 1685^T as *Rhizobium radiobacter* strains, and proposal of *Beijerinckia doebereineriae* sp. nov. to accommodate *Beijerinckia fluminensis* LMG 2819. *Int J Syst Evol Microbiol* **59**, 2323–2328.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (1980).** Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.
- Sneath, P. H. A. & Cowan, S. T. (1958).** An electro-taxonomic survey of bacteria. *J Gen Microbiol* **19**, 551–565.
- Sneath, P. H. A. & Skerman, V. B. D. (1966).** A list of type and reference strains of bacteria. *Int J Syst Bacteriol* **16**, 1–134.
- The Editorial Board of the Judicial Commission of the International Committee on Nomenclature of Bacteria (1966).** International Code of Nomenclature of Bacteria. *Int J Syst Bacteriol* **16**, 459–490.
- Thompson, J. P. & Skerman, V. B. D. (1979).** *Azotobacteraceae: The Taxonomy and Ecology of the Aerobic Nitrogen-Fixing Bacteria*. London: Academic Press.
- Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010).** Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.