NOTE

Phyllobacterium myrsinacearum (subjective synonym Phyllobacterium rubiacearum) emend.

Joris Mergaert,1 Margo C. Cnockaert1 and Jean Swings1,2

Author for correspondence: Joris Mergaert. Tel.: +32 9 264 5120. Fax: +32 9 264 5092. e-mail: joris.mergaert@rug.ac.be

The taxonomic relationships between Phyllobacterium myrsinacearum LMG 2t2T, Phyllobacterium rubiacearum LMG 1t1T and two Phyllobacterium strains representing a collection of isolates from sugar-beet roots were investigated by means of fatty acid analysis and DNA–DNA hybridization. The strains showed very similar fatty acid compositions and more than 96% DNA reassociation to each other. On the basis of these results and available data from the literature, it is proposed that P. rubiacearum be classified as a junior subjective synonym of P. myrsinacearum, and that the sugar-beet isolates be included in the latter species. An emended description of P. myrsinacearum is given.

Keywords: Phyllobacterium myrsinacearum, Phyllobacterium rubiacearum

The genus Phyllobacterium was originally described by Knösel (1962) as including bacteria that develop within leaf nodules of tropical ornamental plants. The genus was revived by Knösel (1984). Two species, the type species, Phyllobacterium myrsinacearum (from Ardisia leaf nodules), and Phyllobacterium rubiacearum (from Pavetta leaf nodules), were defined on the basis of plant source, nitrate reduction and flagellar characteristics. However, conflicting results have been obtained by Lambert et al. (1990) with regard to the nitrate-reduction capability. It has become evident that these bacteria are very common on the rhizoplane and phylloplane of many plants. Lambert et al. (1990) isolated a large number of Phyllobacterium strains from sugar-beet roots, but were unable to assign them to one of the existing species. The phyllobacteria from Ardisia, Pavetta and sugar-beet roots were phenotypically very similar (Lambert et al., 1990), and the 16S rRNA gene sequences of the type strains of P. myrsinacearum (EMBL accession no. D12789) and P. rubiacearum (EMBL accession no. D12790) differ from each other in only two nucleotides (Yanagi & Yamasato, 1993). The sugar-beet isolates showed whole-cell protein-electrophoretic patterns that were almost identical to that of the type strain of P. rubiacearum and that were very similar to those of P. myrsinacearum strains (Lambert et al., 1990). All these observations suggest that these strains might belong to a single species.

To investigate this possibility, P. myrsinacearum LMG 2t2T and P. rubiacearum LMG 1t1T were characterized by using fatty acid analysis and DNA–DNA hybridization. For comparison, two representative strains, LMG 8225 and LMG 8229 (BCCM/LMG Bacteria Collection, Universiteit Gent, Belgium), isolated from sugar-beet roots by Lambert et al. (1990), were included.

For the fatty acid analysis, the strains were grown for 24 h on tryptic soy broth (BBL) solidified with 1.5% agar (Difco), or for 48 h on TY medium (Jarvis et al., 1996). Their fatty acid methyl esters were extracted and separated by GLC using the MIDI system (Microbial ID), as described previously (Mergaert et al., 1993). Essentially the same fatty acids were encountered in extracts prepared on either medium, though the ratios differed. The four strains, when grown on the same medium, showed only minor differences in their fatty acid compositions (Table 1).

DNA was prepared from cells grown on nutrient agar (Oxoid) at 28 °C, according to the method of Pitcher et al. (1989). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells, as described by Willems et al. (2001), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the...
fluorescence measurements. The hybridization temperature was 50 °C. This temperature represents stringent conditions for *Phyllobacterium*, for which the optimal renaturation temperature in the presence of 50% formamide is 42 °C (see Willems *et al.*, 2001). Reciprocal experiments were performed for every pair of strains, and the data given are means of the two values. The DNA binding between *P. myrsinacearum* LMG 2tT and *P. rubiacearum* LMG 1tT was 97%, and these strains showed 96 and 100% DNA binding, respectively, to *Phyllobacterium* sp. LMG 8229. The differences between reciprocal experiments were respectively 8, 16 and 12%.

The high DNA binding values between *P. myrsinacearum* LMG 2tT and *P. rubiacearum* LMG 1tT indicate that they should be classified within a single species, i.e. *P. myrsinacearum* (subjective synonym *P. rubiacearum*), which also includes the sugar-beet isolates from Lambert *et al.* (1990). This is supported by the high phenotypic (Lambert *et al.*, 1990) and chemotaxonomic (Table 1) similarity of these strains.

The unification of the two *Phyllobacterium* species and the inclusion of the sugar-beet isolates into *P. myrsinacearum* necessitates an emendation of the description of the species, given below.

**Emended description of *Phyllobacterium myrsinacearum* (ex Knösel 1962) Knösel 1984**


The description is as given by Knösel (1984) and by Lambert *et al.* (1990), with the following modifications. The cells are motile by means of polar, subpolar or lateral flagella. Nitrate is reduced. Found in leaf nodules of tropical ornamental plants (species of *Myrsinaceae* and *Rubiaeeae*) and on the phylloplane and rhizoplane of other plants. The main fatty acid extracted from cells grown on tryptic soy broth agar is 18:1ω7c while, in extracts from cells grown on TY medium (Jarvis *et al.*, 1996), large amounts of 19:0 cyclo ω8c and 16:0 3OH are also present. The G+C content of the DNA of three strains is 60.3–61.3 mol% (De Smedt & De Ley, 1977).

The type strain is strain LMG 2tT (= ATCC 43590T = NCIMB 12127T = DSM 5892T) and the EMBL accession no. of its 16S rRNA gene sequence is D12789.

**Acknowledgements**

The authors are indebted to the Bijzonder Onderzoeksfonds (Belgium) for personnel grants.

**References**


Lambert, B., Joos, H., Dierickx, S., Vantomme, R., Swings, J.,


