NOTE

***Phyllobacterium myrsinacearum* (subjective synonym *Phyllobacterium rubiacearum*) emend.**

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The taxonomic relationships between *Phyllobacterium myrsinacearum* LMG 2t2^T, *Phyllobacterium rubiacearum* LMG 1t1^T 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 2t2^T and *P. rubiacearum* LMG 1t1^T 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

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The EMBL accession number for the 16S rRNA gene sequence of strain LMG 2t2^T (= ATCC 43590^T = NCIMB 12127^T = DSM 5892^T) is D12789.

Table 1. Fatty acid compositions of *Phyllobacterium* strains

Values are percentages of total fatty acids. TSBA and TY refer to extracts from cells grown for 24 h on tryptic soy broth agar or from cells grown for 48 h on TY medium (Jarvis *et al.*, 1996). Summed feature 2 comprises any combination of 12:0 aldehyde, 14:0 3OH and 16:1 iso I (capital letter refers to the unknown position of the double bond). Summed feature 3 comprises either 15:0 iso 2OH or 16:1 ω 7c or both. ND, Not detected.

Fatty acid	<i>P. myrsinacearum</i> LMG 2t2 ^T		<i>P. rubiacearum</i> LMG 1t1 ^T		<i>Phyllobacterium</i> sp. LMG 8229		<i>Phyllobacterium</i> sp. LMG 8225
	TSBA	TY	TSBA	TY	TSBA	TY	TY
16:0	6.0	6.1	9.1	7.3	8.4	8.6	7.7
18:0	2.1	1.1	3.3	ND	ND	ND	ND
20:0	4.4	3.8	4.0	5.5	3.7	5.6	5.7
18:1 ω 7c	64.9	39.3	62.0	36.4	61.0	31.6	34.3
11-Methyl 18:1 ω 7c	ND	5.0	ND	4.5	2.3	5.5	4.7
19:0 cyclo ω 8c	4.8	15.8	6.2	20.4	7.3	22.3	21.8
15:0 3OH	ND	1.3	ND	ND	ND	ND	ND
16:0 3OH	5.7	10.2	5.7	10.1	5.4	10.3	11.6
18:0 3OH	ND	2.0	ND	2.3	ND	ND	ND
18:1 2OH	5.2	8.9	4.9	7.9	4.5	7.9	8.0
Summed feature 2	4.7	4.9	4.9	5.6	4.4	4.9	6.2
Summed feature 3	2.3	1.9	ND	ND	3.1	3.3	ND

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 2t2^T and *P. rubiacearum* LMG 1t1^T 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 2t2^T and *P. rubiacearum* LMG 1t1^T 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

Subjective synonym: *Phyllobacterium rubiacearum* (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 *Rubiaceae*) and on the phylloplane and rhizoplane of other plants. The main fatty acid extracted from cells grown on tryptic soy broth agar is 18:1 ω 7c while, in extracts from cells grown on TY medium (Jarvis *et al.*, 1996), large amounts of 19:0 cyclo ω 8c 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 2t2^T (= ATCC 43590^T = NCIMB 12127^T = DSM 5892^T) and the EMBL accession no. of its 16S rRNA gene sequence is D12789.

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References

- De Smedt, J. & De Ley, J. (1977). Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *Int J Syst Bacteriol* **27**, 222–240.
- Jarvis, B. D. W., Sivakumaran, S., Tighe, S. W. & Gillis, M. (1996). Identification of *Agrobacterium* and *Rhizobium* species based on cellular fatty acid composition. *Plant Soil* **184**, 143–158.
- Knösel, D. (1962). Prüfung von Bakterien auf Fähigkeit zur Sternbildung. *Zentbl Bakteriol Parasitenkd Infektionskr Hyg II Abt* **116**, 79–100.
- Knösel, D. H. (1984). Genus IV. *Phyllobacterium* (ex Knösel 1962) nom. rev. (*Phyllobacterium* Knösel 1962, 96). In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 254–256. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Lambert, B., Joos, H., Dierickx, S., Vantomme, R., Swings, J.,

Kerstens, K. & Van Montagu, M. (1990). Identification and plant interaction of a *Phyllobacterium* sp., a predominant rhizobacterium of young sugar beet plants. *Appl Environ Microbiol* **56**, 1093–1102.

Mergaert, J., Verdonck, L. & Kersters, K. (1993). Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int J Syst Bacteriol* **43**, 162–173.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid

extraction of bacterial genomic DNA with guanidinium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA–DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51**, 1315–1322.

Yanagi, M. & Yamasato, K. (1993). Phylogenetic analysis of the family *Rhizobiaceae* and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequencer. *FEMS Microbiol Lett* **107**, 115–120.