

# Reclassification of *Flavobacterium resinovorum* Delaporte and Daste 1956 as *Novosphingobium resinovorum* comb. nov., with *Novosphingobium subarcticum* (Nohynek *et al.* 1996) Takeuchi *et al.* 2001 as a later heterotypic synonym

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The taxonomic status of *Flavobacterium resinovorum* Delaporte and Daste 1956 (Approved Lists 1980) was investigated using a polyphasic approach. The 16S rRNA gene sequence of *F. resinovorum* NCIMB 8767<sup>T</sup> was almost identical to that of the type strain of *Novosphingobium subarcticum* (Nohynek *et al.* 1996) Takeuchi *et al.* 2001, with 99.85 % sequence similarity. The DNA–DNA relatedness value between the type strains of these species was 100 %. Phenotypic comparison based on API 20E, API NE and API ZYM kits demonstrated that the type strains of *F. resinovorum* and *N. subarcticum* were also indistinguishable based on their biochemical properties. On the basis of genotypic and phenotypic evidence, it is therefore proposed to reclassify *Flavobacterium resinovorum* as *Novosphingobium resinovorum* comb. nov., with the type strain NCIMB 8767<sup>T</sup> = ATCC 33545<sup>T</sup> = DSM 7478<sup>T</sup> = LMG 8367<sup>T</sup>, and that *Novosphingobium subarcticum* is a later heterotypic synonym of *Novosphingobium resinovorum*.

The genus *Flavobacterium* was created in 1923 to accommodate Gram-negative, non-spore-forming, yellow-pigmented rods that produce acid weakly from carbohydrates (Holmes *et al.*, 1984). This broad definition has subsequently resulted in the inclusion of many phylogenetically heterogeneous species in the genus. Despite several attempts (Bernardet *et al.*, 1996; Xie & Yokota, 2006), the genus still contains poorly characterized species. The aim of this study is to evaluate the taxonomic properties of *Flavobacterium resinovorum*. The species was initially proposed by Delaporte & Daste (1956) and included in the Approved Lists (Skerman *et al.*, 1980), but it has never been included in comparative taxonomic study.

*F. resinovorum* NCIMB 8767<sup>T</sup> was obtained from the NCIMB and grown and maintained on nutrient agar (NA; Difco) at 25 °C. Its 16S rRNA gene sequence was determined and analysed as described previously (Chun & Goodfellow, 1995; Yi & Chun, 2006). The combination of a BLAST search and pairwise alignment analysis based on

the jPHYDIT program (Jeon *et al.*, 2005) indicated that the 16S rRNA gene sequence of *F. resinovorum* NCIMB 8767<sup>T</sup> was almost identical to that of the type strain of *Novosphingobium subarcticum*, KF1<sup>T</sup> (GenBank accession no. X94102), with 99.85 % sequence similarity (2 nucleotide differences out of 1373 bp). Phylogenetic analysis clearly showed that the phylogenetic position of *F. resinovorum* is within the clade encompassing the genus *Novosphingobium* (data not shown). The next closest phylogenetic neighbour to *F. resinovorum* was *Novosphingobium pentaromativorans*, with 95.98 % 16S rRNA gene sequence similarity to the type strain. Therefore, it is evident from 16S rRNA gene sequence analysis that *F. resinovorum* is a member of the genus *Novosphingobium* and shows a very close phylogenetic relationship to *N. subarcticum*.

Genomic DNA of test strains was extracted and purified according to Yi & Chun (2006). DNA–DNA hybridization was carried out as described as described by De Ley *et al.* (1970) with modifications as described by Huß *et al.* (1983), using a Cary 300 Bio model UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller (Varian). The DNA–DNA relatedness value between *F. resinovorum* NCIMB 8767<sup>T</sup> and *N. subarcticum* KCTC 2890<sup>T</sup> (obtained from the Korean Collection of Type Cultures) was 100 %. The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *F. resinovorum* NCIMB 8767<sup>T</sup> is EF029110.

Details of API test results and fatty acid compositions for *F. resinovorum* NCIMB 8767<sup>T</sup> and *N. subarcticum* KCTC 2890<sup>T</sup> are available as supplementary material with the online version of this paper.

combination of 16S rRNA gene sequence similarity and DNA–DNA relatedness clearly indicates that the two strains belong to the same species.

To elucidate further the taxonomic relationship between *F. resinovorum* and *N. subarcticum*, the phenotypic profile of *N. subarcticum* KCTC 2890<sup>T</sup> was compared with that of *F. resinovorum* NCIMB 8767<sup>T</sup>. Both type strains were grown and characterized in duplicate using the API 20E, API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions. The two strains showed identical results for 60 tests. Two tests (2-naphthyl caprylate utilization in API ZYM and  $\beta$ -galactosidase activity in API 20NE) produced a weakly positive result in one strain with a positive result in the other, and assimilation of caprate (API 20NE) produced variable results for *N. subarcticum* KCTC 2890<sup>T</sup>. Cellular fatty acid compositions of the test strains were compared using the MIDI system as described by Yi & Chun (2006). *F. resinovorum* NCIMB 8767<sup>T</sup> and *N. subarcticum* KCTC 2890<sup>T</sup> showed generally similar profiles, with major amounts of C<sub>18:1</sub>ω7c (45.28 and 59.95 %, respectively). The biochemical and fatty acid profiles of the two strains are given in Supplementary Tables S1 and S2 in IJSEM Online.

On the basis of molecular, chemotaxonomic and physiological comparison, it is clear that *Novosphingobium subarcticum* (Nohynek *et al.* 1996) Takeuchi *et al.* 2001 is a later heterotypic synonym of *Flavobacterium resinovorum* Delaporte and Daste 1956 (Approved Lists 1980). In addition, *Flavobacterium resinovorum* should be transferred to the genus *Novosphingobium* as *Novosphingobium resinovorum* comb. nov.

### Description of *Novosphingobium resinovorum* (Delaporte and Daste 1956) comb. nov.

*Novosphingobium resinovorum* [re.si.no.vo'rum. L. n. *resina* resin or gum of trees; L. v. *vorare* to devour; N.L. neut. adj. *resinovorum* (sic) resin-devouring].

Basonym: *Flavobacterium resinovorum* Delaporte and Daste 1956 (Approved Lists 1980).

Heterotypic synonyms: *Novosphingobium subarcticum* (Nohynek *et al.* 1996) Takeuchi *et al.* 2001, *Sphingomonas subarctica* Nohynek *et al.* 1996.

In addition to the description given by Nohynek *et al.* (1996) for *Sphingomonas subarctica*, the organism shows positive reactions in the following biochemical tests: utilization of citrate, production of acetoin, cytochrome oxidase, catalase (API 20E), 2-naphthyl phosphate, 2-naphthyl butyrate, 2-naphthyl caprylate, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, 2-naphthyl phosphate, naphthol-AS-BI-phosphate, 2-naphthyl  $\alpha$ -D-glucopyranoside, 6-bromo-2-naphthyl  $\beta$ -D-galactopyranoside (API ZYM),  $\beta$ -glucosidase,  $\beta$ -galactosidase and assimilation of glucose, arabinose, N-acetylglucosamine, maltose, malate and citrate (API 20NE). The organism does not react in the following biochemical tests:  $\beta$ -galactosidase, arginine dihydrolase, lysine decarboxylase,

ornithine decarboxylase, H<sub>2</sub>S production, urease, tryptophan deaminase, indole production, gelatinase, fermentation/oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose (API 20E), 2-naphthyl myristate, L-cystyl 2-naphthylamide, N-benzoyl-DL-arginine 2-naphthylamide, N-glutaryl-phenylalanine 2-naphthylamide, 6-bromo-2-naphthyl  $\alpha$ -D-galactopyranoside, 2-naphthyl  $\beta$ -D-galactopyranoside, naphthol-AS-BI- $\beta$ -D-glucuronide, 1-naphthyl N-acetyl- $\beta$ -D-glucosaminide, 6-bromo-2-naphthyl  $\alpha$ -D-mannopyranoside, 2-naphthyl  $\alpha$ -L-fucopyranoside (API ZYM), reduction of nitrates to nitrites/nitrogen, indole production, acidification, arginine dihydrolase, urease, protease and assimilation of mannose, mannitol, gluconate, adipate and phenylacetate (API 20NE).

The type strain is NCIMB 8767<sup>T</sup> = ATCC 33545<sup>T</sup> = DSM 7478<sup>T</sup> = LMG 8367<sup>T</sup>.

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