Reclassification of *Flexibacter aggregans* (Lewin 1969) Leadbetter 1974 as a later heterotypic synonym of *Flexithrix dorotheae* Lewin 1970

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The taxonomic relationship between [*Flexibacter*] *aggregans* IAM 14894^T and *Flexithrix dorotheae* NBRC 15987^T was investigated by means of DNA–DNA hybridization and phenotypic characteristics. On the basis of these results, it is proposed that *Flexibacter aggregans* (Lewin 1969) Leadbetter 1974 be considered a later heterotypic synonym of *Flexithrix dorotheae* Lewin 1970. Emended descriptions of the species *Flexithrix dorotheae* and the genus *Flexithrix* are also given.

Flexibacter aggregans was described by Leadbetter (1974) as a member of genus *Flexibacter*. However, on the basis of phylogenetic analysis, *Flexibacter aggregans* was not included in the cluster of *Flexibacter flexilis*, the type species of the genus *Flexibacter* (Nakagawa *et al.*, 1997), but was taken to represent a separate lineage within the genus *Flexithrix* (Sly *et al.*, 1998). The genus *Flexithrix* was created by Lewin (1970) with a single species, *Flexithrix dorotheae* (Lewin, 1970). Recently, Nakagawa *et al.* (2002) indicated that the 16S rRNA gene sequences of [*Flexibacter*] *aggregans* NBRC 15976^T (GenBank accession no. AB078040) and *Flexithrix dorotheae* NBRC 15987^T (accession no. AB078077) are identical. In the present note, we report on a polyphasic taxonomic study that demonstrates that [*Flexibacter*] *aggregans* and *Flexithrix dorotheae* belong in the same species; therefore, we propose the synonymy of *Flexithrix dorotheae* and *Flexibacter aggregans*.

The type strains of [*Flexibacter*] *aggregans*, IAM 14894^T, and *Flexithrix dorotheae*, NBRC 15987^T, were respectively obtained from the IAM Culture Collection, Japan, and the NITE Biological Resource Center, Japan (Table 1). The 16S rRNA gene sequences of these strains were analysed as described by Hosoya *et al.* (2006). Sequences were compared by BLAST 2 SEQUENCES (Tatusova & Madden, 1999). Alignment gaps and unidentified base positions were not taken into consideration in the calculation. The newly determined 16S rRNA gene sequences of [*Flexibacter*] *aggregans* IAM 14894^T (GenBank accession no. AB288294) and *Flexithrix dorotheae* NBRC 15987^T (data not shown) were identical. In addition, the 16S rRNA gene sequence determined in this study for *Flexithrix dorotheae* NBRC 15987^T was identical to that determined previously for *Flexithrix dorotheae* ATCC

23163^T (GenBank accession no. AB288293) and the 16S rRNA gene sequence of [*Flexibacter*] *aggregans* IAM 14894^T was almost identical to that determined previously for [*Flexibacter*] *aggregans* ATCC 23162^T (GenBank accession no. M64628).

Genomic DNA was extracted by the method of Saito & Miura (1963). For analysis of relatedness, DNA–DNA hybridization was carried out at 40 °C and measured fluorometrically using the method of Ezaki *et al.* (1989). The DNA G + C content was determined by HPLC according to the method of Mesbah *et al.* (1989). The respiratory quinone was analysed by HPLC (Komagata & Suzuki, 1987). For analysis of cellular fatty acids, cells were grown for 48 h at 30 °C on trypticase soy agar (TSA; BBL) supplemented with 0.5 × artificial seawater (ASW; 1 × ASW consists of 3 % NaCl, 0.07 % KCl, 1.08 % MgCl₂·6H₂O, 0.54 % MgSO₄·7H₂O and 0.1 % CaCl₂·2H₂O) and fatty acids were analysed using the GC-based Microbial Identification system (MIDI). Growth at different temperatures (8–45 °C) and different pH values (pH 5–9) was tested on SP5 agar (containing 0.9 % casitone, 0.1 % yeast extract, 0.5 × ASW and 1.5 % agar). Salt tolerance was tested on R2A agar (Difco), supplemented with 0–10 % NaCl. Oxidase activities were tested by spreading oxidase test paper (Nissui) with cell pellets. Catalase activities were tested using a 3 % H₂O₂ solution. Degradation of casein and starch was tested according to Smibert & Krieg (1994). Degradation of Tweens 20, 40, 60 and 80 and L-tyrosine was tested according to Barrow & Feltham (1993). Degradation of DNA was tested on DNA agar (Nissui). Decomposition of alginate was tested using basal seawater medium, containing 0.1 % peptone, 0.1 % NH₄Cl, 0.0075 % KH₂PO₄·3H₂O, 0.0028 % FeSO₄·7H₂O, 0.61 % Tris base, 2 % alginate and 1 % agar. Hydrolysis of agar and CM-cellulose was tested by inoculation of stab cultures prepared using SP5 medium

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of [*Flexibacter*] *aggregans* IAM 14894^T is AB288294.

Table 1. Strains included in this study

Strain	Received as:	Strain history	Isolation source
NBRC 15987 ^T	<i>Flexithrix dorotheae</i>	← IFO 15987 ^T ← DSM 6795 ^T ← H. Reichenbach, Ft d1 ^T ← ATCC 23163 ^T ← R. A. Lewin, QQ3 ^T	Marine silt, Kerala, India
IAM 14894 ^T	<i>Flexibacter aggregans</i>	← ATCC 23162 ^T ← R. A. Lewin, NN13 ^T	Green-brown sand, Canoe Beach, Tema, Ghana

containing 1 % agar and 3 % CM-cellulose, respectively. These tests were carried out using medium supplemented with 0.5 × ASW. Acid production from sugars was assessed using modified O/F medium (Smibert & Krieg, 1994). Tests with the commercial systems API ZYM and API 20E (bioMérieux) were generally performed according to the manufacturer's instructions. Cells for inoculation to the API test strips were suspended in 0.5 × ASW. The API ZYM tests were read after 4 h incubation at 30 °C and API 20E tests after 48 h incubation at 30 °C.

Results of phenotypic characteristics are given in the emended species description. [*Flexibacter*] *aggregans* IAM 14894^T and *Flexithrix dorotheae* NBRC 15987^T showed nearly the same characteristics; the only difference was the degradation of tyrosine by [*Flexibacter*] *aggregans* IAM 14894^T. The 16S rRNA gene sequences of the type strains of [*Flexibacter*] *aggregans* and *Flexithrix dorotheae* were identical. The cellular fatty acid compositions of the strains were almost the same; the major components were 16:1ω5c and iso-15:0 (Table 2). The G + C content of these strains was the same, at 35.6 mol%. The major isoprenoid quinone of both strains was MK-7. Moreover, high DNA–DNA

relatedness values (74–107 %) between [*Flexibacter*] *aggregans* IAM 14894^T and *Flexithrix dorotheae* NBRC 15987^T indicated that these strains should be classified within a single species. This conclusion is supported by their phenotypic similarity. According to Rules 15 and 17 of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1992), it is proposed that *Flexibacter aggregans* is a later heterotypic synonym of *Flexithrix dorotheae*. Therefore, an emended description of the genus *Flexithrix* is given below.

Emended description of the genus *Flexithrix* Lewin 1970

Flexithrix (Fle'xi.thrix. L. adj. *flexilis* flexible; Gr. n. *thrix* hair; N.L. fem. n. *Flexithrix* flexible hair).

The morphological characteristics of this genus are described by Reichenbach (1989). Gram-negative. Chemo-organotrophs. Strict aerobes. Require NaCl for growth. Motile by gliding. Positive for oxidase, catalase, alkaline phosphatase and acid phosphatase. Predominant fatty acids are 16:1ω5c and iso-15:0. The respiratory quinone is MK-7. This genus belongs to the phylum *Bacteroidetes*. The type species is *Flexithrix dorotheae*.

Emended description of *Flexithrix dorotheae* Lewin 1970

Flexithrix dorotheae (do.ro.the'ae. N.L. gen. fem. n. *dorotheae* of Dorothy, named to commemorate a deceased technical assistant, Dorothy White).

Main characteristics are described by Reichenbach (1989). In addition, the growth temperature is 17–40 °C; no growth occurs at either 8 or 45 °C. The pH range for growth is 6.5–8.0. Growth occurs at NaCl concentrations of 2–5 %. Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, *N*-acetyl-β-glucosamidase and α-fucosidase and for degradation of CM-cellulose, DNA and Tweens 20, 40, 60 and 80. Degradation of tyrosine is variable. Does not decompose agar, alginate, casein, citrate, starch or urea. Does not reduce nitrate. No production of acetoin, H₂S or indole. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, lipase (C4), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and α-mannosidase. Acid is produced from arabinose, cellobiose, fructose, galactose, glucose, lactose,

Table 2. Fatty acid profiles of the type strains of [*Flexibacter*] *aggregans* and *Flexithrix dorotheae*

Values are percentages of total fatty acids. tr, Trace.

Fatty acid	[<i>Flexibacter</i>] <i>aggregans</i> IAM 14984 ^T	<i>Flexithrix dorotheae</i> NBRC 15987 ^T
iso-11:0	tr	1.0
14:0	1.1	1.5
iso-15:0	19.2	19.9
15:0	tr	1.0
Summed feature 3*	11.3	9.9
16:1ω5c	37.6	37.7
16:0	5.1	4.9
iso-15:0 3-OH	3.3	4.0
Summed feature 4†	2.7	1.0
iso-17:0	2.4	tr
16:0 3-OH	1.5	2.8
iso-17:0 3-OH	7.4	7.1

*Summed feature 3 contains iso-15:0 2-OH and/or 16:1ω7c.

†Summed feature 4 contains anteiso-17:1 B and/or iso-17:1 I.

maltose, mannose, raffinose, rhamnose, sucrose, trehalose and xylose. Does not produce acid from dulcitol, glycerol, inositol, mannitol or sorbitol. The DNA G + C content of the type strain is 35.6 mol%.

The type strain is NBRC 15987^T (=DSM 6795^T=ATCC 23163^T).

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