Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov.

P. L. Steyn,¹ P. Segers,² M. Vancanneyt,² P. Sandra,³ K. Kersters² and J. J. Joubert⁴

Author for correspondence: P. Segers. Tel: +32 9 2645115. Fax: +32 9 2645346.
e-mail: Paul.Segers@rug.ac.be

¹ Departement
Mikrobiologie en
Plantpatologie,
Universiteit van Pretoria,
0002 Pretoria, South Africa

² Laboratorium voor
Microbiologie, Universiteit
Gent, B-9000 Gent,
Belgium

³ Laboratorium voor
Organische Scheikunde,
Universiteit Gent, B-9000
Gent, Belgium

⁴ Departement
Geneeskundige
Mikrobiologie, Universiteit
van Stellenbosch, Posbus
19063, 7505 Tygerberg,
South Africa

Sixteen heparinase-producing isolates, related to *Sphingobacterium heparinum*, were grouped into three major clusters by SDS-PAGE and DNA-rRNA hybridizations. Based on a polyphasic approach, it was shown that isolates of two of these clusters and *S. heparinum* species belong to a new genus for which the name *Pedobacter* is proposed. The genus consists of *Pedobacter heparinus* comb. nov. (formerly *Sphingobacterium heparinum*), which is the type species, *Pedobacter piscium* comb. nov. (formerly *Sphingobacterium piscium*), *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. and four as-yet-unnamed DNA hybridization groups. All the previously named taxa can be discriminated by phenotypic features, but have strong overall similarities with representatives of the genus *Sphingobacterium* and the misclassified species [*Flexibacter*] *canadensis*. All these organisms constitute a separate rRNA branch in rRNA superfamily V for which the family *Sphingobacteriaceae* fam. nov. is proposed.

Keywords: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov., *Pedobacter saltans* sp. nov., *Sphingobacteriaceae* fam. nov.

INTRODUCTION

Sphingobacterium heparinum, represented by a single isolate, remained for many years the sole representative of a strictly aerobic Gram-negative, heparinase-producing bacterium. Joubert (14) succeeded in obtaining heparinase-producing isolates from soil and activated sludge. Sixteen isolates were investigated by SDS-PAGE of whole-cell proteins and found to consist of three discernable groups (26). One group comprising 11 isolates and two subcultures of the type strain of *S. heparinum* were found to cluster into a single group (consisting of two sub-groups Ia and Ib). The second group (II) and a third group (III) contain one and four

isolates, respectively. Corresponding rRNA groups and sub-groups were found by DNA-rRNA hybridizations (26, 27).

The generic status of *S. heparinum* has been discussed repeatedly and the organism has been successively transferred to other genera. The species was first described as *Flavobacterium heparinum* (20), and was later reclassified in the genus *Cytophaga* as *Cytophaga heparina* (2). Reichenbach (21) has already questioned the inclusion of the organism in the genus *Cytophaga*. More recently the phylogenetic position of *C. heparina* was further elucidated (7, 8, 17, 18, 19, 26). Based on phenotypic and chemotaxonomic analyses, Takeuchi & Yokota (28) proposed the transfer of *C. heparina* to the genus *Sphingobacterium* as *S. heparinum*. The genus *Sphingobacterium* is part of rRNA superfamily

Abbreviation: FAME, fatty acid methyl ester.

Table 1. Strains used, some other strain designations and source of isolation

LMG, Laboratorium voor Microbiologie Gent Culture Collection, University of Gent, Belgium; ATCC, American Type Culture Collection, Rockville, MD, USA; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Sweden; IFO, Institute for Fermentation, Osaka, Yodogawa-ku, Osaka 532, Japan.

Name and LMG no.*	Other designation(s)	Source
<i>Pedobacter heparinus</i> (Ia1)		
LMG 10399 ^T	ATCC 13125 ^T , IFO 12017 ^T	Dry soil
LMG 10344 ^{T†}	AL [†]	Dry soil
<i>Pedobacter africanus</i> (Ia3)		
LMG 10353 ^T		Belfast, Eastern Transvaal, South Africa; soil
LMG 10345		Windhoek, Namibia; activated sludge
LMG 10346		Pretoria, South Africa; soil
LMG 10347		Western Caprivi, Namibia; soil
LMG 10348		Pretoria, South Africa; activated sludge
LMG 10349		Eastern Kavango, Namibia; soil
<i>Pedobacter piscium</i>		
LMG 14023 ^T	IFO 14985 ^T	—
LMG 14024	IFO 14984	—
<i>Pedobacter saltans</i> (III)		
LMG 10337 ^T		Iceland; soil
LMG 10338		Iceland; soil
LMG 10340		Brussels, Belgium; soil
LMG 10341		Rüdesheim, Germany; soil
<i>Pedobacter</i> sp. (Ia2)		
LMG 9525	LMG 10350	Ephesus, Turkey; soil
LMG 10343		Swartkoppies, Transvaal, South Africa; soil
<i>Pedobacter</i> sp. (Ib1)		
LMG 9527	LMG 10352	Langkloof, Eastern Cape, South Africa; soil
LMG 10354		Roodeplaat, Pretoria, South Africa; soil
<i>Pedobacter</i> sp. (Ib2)		
LMG 10342		Sambyu, Kavango, Namibia; soil
<i>Pedobacter</i> sp. (II)		
LMG 10351		Amsterdam, The Netherlands; soil
<i>Sphingobacterium spiritivorum</i>		
LMG 8347 ^T	CCUG 13224 ^T , IFO 14948 ^T	Kansas, USA; intra-uterine device
LMG 8348	CCUG 15910	—
<i>Sphingobacterium multivorum</i>		
LMG 8342 ^T	CCUG 11736 ^T , IFO 14947 ^T	Spleen
LMG 8354	CCUG 22177	Denmark; soil
<i>Sphingobacterium mizutae</i>		
LMG 8340t1 ^T	CCUG 15907 ^T , IFO 14946 ^T	Ventricular fluid of foetus
LMG 8341	CCUG 15908	—
<i>Sphingobacterium thalpophilum</i>		
LMG 11520 ^T	CCUG 22397 ^T , IFO 14963 ^T	Wound swab
LMG 11521	CCUG 22398	Human abscess
<i>Sphingobacterium faecium</i>		
LMG 14022 ^T	IFO 15299 ^T	—
[<i>Flavobacterium</i>] <i>yabuuchiae</i>		
LMG 11523 ^T	CCUG 26765 ^T , IFO 14975 ^T	USA; human sputum
[<i>Flexibacter</i>] <i>canadensis</i>		
LMG 8368 ^T	ATCC 29591 ^T	Soil

* The SDS-PAGE or DNA hybridization sub-group number (26, 27 and this paper) are shown in parentheses.

† Strain LMG 10344^T is an independently received subculture (from Dr A. Linker, Salt Lake City, UT, USA) of the type strain.

V, the so-called *Cytophaga-Flavobacterium-Bacteroides* group (1, 23, 26). The confused taxonomy of this large group of bacteria was recently clarified by rRNA similarity studies (1, 7, 8, 17, 18, 19) and quinone analyses (5, 17, 18, 28). This resulted in the creation of new genera (18, 30) and emended descriptions of the genus *Flavobacterium*, the family *Flavobacteriaceae* (1) and the genus *Cytophaga* (18).

This paper reports on the classification of the heparinase-producing isolates by a more detailed phenotypic description and determination of fatty acid content of the obtained clusters and related organisms, together with some additional DNA-rRNA and DNA-DNA hybridizations. Preliminary data have already been presented by Steyn *et al.* (27). A new genus *Pedobacter*, encompassing two former *Sphingobacterium* species, *Pedobacter heparinus* comb. nov. and *Pedobacter piscium* comb. nov., and two new species, *Pedobacter africanus* and *Pedobacter saltans*, is proposed. These names will be used throughout this paper. Several strains with a separate genotypic position will be classified and can be considered as unnamed genomic species of *Pedobacter*. We also propose the family *Sphingobacteriaceae* comprising the newly described organisms, the genus *Sphingobacterium* and [*Flexibacter*] *canadensis*. Square brackets are used to indicate generically misclassified bacteria.

METHODS

Micro-organisms and growth conditions. Strains studied are listed in Table 1. Information about the bacterial strains, considered in this paper, and growth conditions were given before (26, 27, 28, 32).

Conventional tests. The conventional tests were done according to Cowan (4) or Sonnenwirth (25), unless stated otherwise. The oxidation/fermentation test was done in SIM medium (Merck). Acid and gas production from glucose, lactose and sucrose were determined in Kligler Iron agar (Difco Laboratories). Cytochrome-*c* oxidase was determined by adding a few drops of tetramethyl-*p*-phenylenediamine solution to a 5-d-old Tryptose agar (Biolab) slant. Lysine deaminase and lysine decarboxylase were determined in Lysine iron agar (Biolab). Utilization of citrate and mannitol was determined on Citrate mannitol agar (Biolab). Urease activity was determined in Urease agar base (Difco) to which 1.5% agar was added. For the determination of acid production from inositol and arabinose a sugar base consisting of 2% Bacto peptone (Difco) and 0.5% NaCl was used, to which 2.5% inositol or arabinose was added. Nitrate reduction was determined on Sellers agar (Difco). The medium described by Cowan (1979) was used for the determination of ornithine decarboxylase. The isolates were also tested for their ability to grow on MacConkey agar (Difco). The inoculated tubes were incubated at 25 °C and read after 7 and 14 d, respectively. Positive and negative controls were included in all tests. Heparinase production was determined by the method of Joubert *et al.* (13).

Carbon assimilation tests. The API 50CH, 50AO, 50AA (API Systems) galleries were used according to a standardized procedure (15).

API 20E and API ZYM micromethod tests. These tests were done according to the instructions of the manufacturer (API

Systems). A suspension was made of a 3-d-old slant in sterile water, adjusted to give a reading of 5–6 on the MacFarland scale and used for inoculating the galleries. The API 20E galleries were incubated at 25 °C for 7 d, and the API ZYM galleries at 30 °C for 4 h.

GC of cellular fatty acids. The methods using the MIS system (Microbial Identification System) have been described previously (27). Fatty acid methyl esters (FAMES) were separated on an apolar fused silica capillary column coated with a phenyl methyl silicone gum phase (Hewlett-Packard). Under these conditions the fatty acids 16:1 ω 7c and iso-15:0(2-OH), two major components of the taxa studied, eluted for most strains as a single peak. To quantify both FAMES a second analysis was performed on a polar cyanopropyl silicone column (31) where both peaks elute at clearly different retention times.

DNA-rRNA and DNA-DNA hybridizations. DNA-rRNA and DNA-DNA hybridizations were performed as described previously (26, 27).

RESULTS

DNA-rRNA hybridization

Previous data (26, 27) together with results from this study (Table 2), are presented in Fig. 1 as a simplified dendrogram based on the thermal stability of the hybrids [$T_{m(e)}$ values]. The overall groupings are similar to those described previously (26, 27). The heparinase-producing bacteria are situated on a separate rRNA branch, the *Pedobacter* branch, consisting of two sub-branches, the *P. heparinus* sub-branch and the *P. saltans* sub-branch. Several genotypic sub-groups and *S. piscium* are situated on the *P. heparinus* sub-branch. *Sphingobacterium* species and [*Flexibacter*] *canadensis* are the nearest relatives to the *Pedobacter* branch.

DNA-DNA hybridization

Hybridizations were performed between and within each of the rRNA groups or corresponding protein electrophoretic groups. The results are shown in Table 3. The *P. heparinus* sub-branch (RNA group I, 26) is shown to split up into seven genotypic sub-groups. Five of these genomic groups belong to the protein clusters Ia and Ib (26): *P. heparinus* (Ia1), *P. africanus* (Ia3), and three unnamed *Pedobacter* genomic species (Ia2, Ib1 and Ib2). *Pedobacter* strain LMG 10351 (group II) and *P. piscium*, also located on this *P. heparinus* rRNA sub-branch, showed no significant DNA similarity with the former five sub-groups. The protein electrophoretic group III strains (*P. saltans* sub-branch) constitute a separate and homogeneous group.

GC of cellular fatty acids

Table 4 shows the cellular fatty acid content of the heparinase-producing taxa (sub-groups) and of the *Sphingobacterium* species. Dominant fatty acids for all strains were: iso-15:0, iso-15:0(2-OH), iso-15:0(3-

Table 2. DNA base compositions of strains and $T_{m(e)}$ values of DNA-rRNA hybrids

Some data are from references (23), (26) and (27).

Name*	LMG no.	G + C content (mol %)	$T_{m(e)}$ in °C with ³ H-labelled 23S rRNA of		
			<i>P. heparinus</i> LMG 10339 ^T	<i>P. saltans</i> LMG LMG 10337 ^T	<i>S. spiritivorum</i> LMG 8347 ^T
<i>Pedobacter heparinus</i> (Ia1)	10339 ^T	43.0	80.0	70.4	67.1
<i>Pedobacter heparinus</i> (Ia1)	10344 ^T	42.3	80.1		
<i>Pedobacter</i> sp. (Ia2)	9525	42.9	79.3		
<i>Pedobacter</i> sp. (Ia2)	10343	42.3	78.9		
<i>Pedobacter africanus</i> (Ia3)	10353 ^T	44.2	77.8		
<i>Pedobacter africanus</i> (Ia3)	10348	44.1	78.2	70.9	
<i>Pedobacter africanus</i> (Ia3)	10349	43.7	77.9		
<i>Pedobacter</i> sp. (Ib1)	9527	37.5	77.0	70.9	67.4
<i>Pedobacter</i> sp. (Ib1)	10354	37.4	76.8		67.4
<i>Pedobacter</i> sp. (Ib2)	10342	39.5	77.4		
<i>Pedobacter piscium</i>	14023 ^T	40.4	75.3	70.3	68.9
<i>Pedobacter piscium</i>	14024	41.0	75.5	70.2	69.3
<i>Pedobacter</i> sp. (II)	10351	41.2	74.2	71.6	
<i>Pedobacter saltans</i> (III)	10337 ^T	37.0	70.9	81.2	67.6
<i>Pedobacter saltans</i> (III)	10338	36.9	70.5	81.2	
<i>Pedobacter saltans</i> (III)	10341	37.1	70.6	80.9	
<i>Sphingobacterium spiritivorum</i>	8347 ^T	39.8	69.5	68.5	77.2
<i>Sphingobacterium spiritivorum</i>	8348	39.8			76.7
<i>Sphingobacterium multivorum</i>	8342 ^T	40.5	67.4	68.7	72.6
<i>Sphingobacterium multivorum</i>	8354	39.9			73.0
<i>Sphingobacterium mizutae</i>	8340t1 ^T	39.3	67.3	69.5	71.2
<i>Sphingobacterium mizutae</i>	8341	40.0			72.3
<i>Sphingobacterium thalpophilum</i>	11520 ^T	44.0	65.2		69.0
<i>Sphingobacterium thalpophilum</i>	11521	44.2			68.2
<i>Sphingobacterium faecium</i>	14022 ^T	37.3	67.3	69.0	73.5
[<i>Flavobacterium</i>] <i>yabuuchiae</i>	11523 ^T	38.1			76.6
[<i>Flexibacter</i>] <i>canadensis</i>	8368 ^T	38.4	67.9		

* The SDS-PAGE or DNA hybridization sub-group numbers are shown in parentheses (see Table 1).

OH), 16:0, 16:1 ω 7c and iso-17:0(3-OH). As discussed below, only minor qualitative differences among the different genera and species are found. Characteristic for all representatives of the newly proposed family *Sphingobacteriaceae* is the presence of significant amounts of the fatty acids iso-15:0(2-OH) and 16:1 ω 7c (both comprised into summed feature 4, MIS version 3.9). As explained above, using the MIS GC conditions both FAMES have very similar retention times and in several cases elute as a single peak. Using a more polar chromatographic system, we proved that iso-15:0(2-OH) and 16:1 ω 7c occur in all of the taxa investigated. Our data confirmed that presented by Yabuuchi & Moss (33), Yabuuchi *et al.* (32), Dees *et al.* (5), and Takeuchi & Yokota (28).

Phenotypic features

The results of an extensive phenotypic analysis and sensitivity study to antibiotics, with descriptive and discriminative information, are presented in Tables 5 and 6. [*Flavobacterium*] *yabuuchiae* and [*Flexibacter*]

canadensis were not tested. All strains studied produce on most agar media a yellow or creamy white non-fluorescent pigment, and they produce catalase, oxidase, phosphatase, esterase lipase (C8), leucine arylamidase, α -glucosidase and *N*-acetyl- β -glucosaminidase. Of the 49 carbohydrates tested (Table 5), all strains assimilate the carbohydrates D-glucose, D-mannose and amygdalin, and 11 of these compounds are not utilized. Very few organic acids are utilized. Several *Pedobacter* strains and one *Sphingobacterium mizutae* strain can utilize pyruvate. Of the 49 amino acids and amines tested, glucosamine is utilized by all strains, whereas 12 of these substrates are utilized by some strains (Table 5).

DISCUSSION

Proposal of *Pedobacter* gen. nov.

The genus *Sphingobacterium* was created by Yabuuchi *et al.* (32) to contain two former *Flavobacterium* species (10, 11), *Sphingobacterium spiritivorum* and *Sphingo-*

[illegible]

linked to the heparinase-producing bacteria at a mean $T_{m(e)}$ of about 68 °C (Fig. 1). These data justify the creation of the new genus *Pedobacter* for the heparinase-producing isolates. The *Pedobacter* branch splits into two sub-branches at $T_{m(e)} = 70.9$ °C. Although the *Pedobacter saltans* sub-branch can be considered to constitute a separate genus based on the large $T_{m(e)}$ difference, it is provisionally classified in the genus *Pedobacter* because of the limited number of strains available (four), and the lack of differentiating phenotypic features. Furthermore, we propose to reclassify *S. heparinum* and *S. piscium*, with newly isolated heparinase-producing taxa, in the genus *Pedobacter*. *Sphingobacterium* still remains genotypically a rather heterogeneous genus [$T_{m(e)}$ span of 8 °C], with *S. thalophilum* as the most remote species; however, a lack of distinctive features (Table 5; 28) of its members justifies for the moment the current classification.

169

Table 4. Mean relative fatty acid composition of *Pedobacter* and *Sphingobacterium* taxa

Standard deviations are shown in parentheses; TR, trace (<1.0%); –, not detected. Abbreviations: *Fa.*, *Flavobacterium*; *F.*, *Flexibacter*; *P.*, *Pedobacter*; *S.*, *Sphingobacterium*; ECL, equivalent chain length.

Name*	ECL 13:566	14:0	15:0	15:1 ω 6c	iso- 15:0	anteiso- 15:0	iso- 15:0(2-OH)	iso- 15:0(3-OH)	16:0
<i>P. heparinus</i> (Ia1)	5.0 (2.9)	1.1 (0.1)	1.1 (0.1)	–	28.2 (3.7)	TR	10.4 (0.3)	2.5 (0.1)	3.0 (0.4)
<i>P. africanus</i> (Ia3)	2.6 (0.5)	1.5 (0.4)	TR	TR	26.6 (2.6)	TR	10.4 (1.2)	2.1 (0.8)	3.8 (1.3)
<i>P. piscium</i>	TR	1.3 (0.1)	–	–	26.2 (0.3)	2.6 (0.5)	10.7 (0.5)	2.5 (0.4)	3.3 (0.6)
<i>P. saltans</i> (III)	1.1 (0.2)	TR	1.4 (0.3)	1.4 (0.2)	31.4 (2.6)	2.9 (0.4)	8.9 (1.6)	2.8 (0.3)	4.0 (0.7)
<i>Pedobacter</i> sp. (Ia2)	1.6 (0.3)	1.2 (0.3)	TR	TR	27.2 (3.5)	TR	9.5 (1.2)	3.1 (0.1)	2.2 (1.1)
<i>Pedobacter</i> sp. (Ib1)	2.1 (0.6)	1.0 (0.1)	TR	TR	25.4 (6.7)	TR	9.9 (1.2)	2.5 (0.3)	3.3 (2.8)
<i>Pedobacter</i> sp. (Ib2)	1.7	1.6	–	–	28.4	1.5	7.1	2.4	3.8
<i>Pedobacter</i> sp. (II)	2.0	TR	1.4	TR	38.3	1.3	15.4	2.4	1.1
<i>S. faecium</i>	TR	TR	–	–	24.6	TR	15.9	3.7	4.5
<i>S. mizutae</i>	1.3 (0.7)	TR	–	–	30.0 (6.2)	TR	25.6 (8.2)	3.0 (1.1)	TR
<i>S. multivorum</i>	–	2.7 (1.6)	–	–	22.2 (3.6)	–	17.4 (0.9)	3.2 (1.1)	7.8 (2.8)
<i>S. spiritivorum</i>	TR	1.0 (0.4)	–	–	30.1 (2.0)	TR	21.5 (0.3)	2.2 (0.3)	3.5 (0.1)
<i>S. thalophilum</i>	1.4 (0.0)	3.2 (0.2)	–	–	17.7 (0.1)	–	24.6 (1.3)	4.3 (0.2)	6.0 (0.9)
[<i>Fa.</i>] <i>yabuuchiae</i>	TR	TR	–	–	30.4	2.3	20.6	2.2	3.2
[<i>F.</i>] <i>canadensis</i> †	TR	TR	2.2	1.8	35.7	4.9	ND‡	3.5	2.6
	16:1 ω 5c	16:1 ω 7c	16:0 10 methyl	16:0 (2-OH)	16:0 (3-OH)	ECL 16:580	iso- 17:1 ω 9c	anteiso- 17:1 ω 9c	iso- 17:0(3-OH)
<i>P. heparinus</i> (Ia1)	1.4 (0.2)	20.2 (0.5)	–	–	1.5 (0.1)	1.0 (0.1)	6.3 (0.6)	–	15.2 (0.8)
<i>P. africanus</i> (Ia3)	2.1 (0.3)	23.7 (3.5)	–	TR	3.1 (0.7)	1.0 (0.2)	4.4 (1.2)	–	14.7 (2.2)
<i>P. piscium</i>	3.5 (0.4)	31.4 (1.9)	–	–	4.5 (0.7)	TR	1.6 (0.2)	1.2 (0.3)	9.2 (1.4)
<i>P. saltans</i> (III)	TR	19.6 (1.5)	–	–	TR	1.2 (0.1)	6.6 (1.4)	–	12.7 (0.6)
<i>Pedobacter</i> sp. (Ia2)	4.0 (1.9)	24.5 (6.2)	–	–	1.2 (0.7)	TR	6.0 (2.7)	–	15.3 (0.5)
<i>Pedobacter</i> sp. (Ib1)	4.5 (3.0)	26.1 (4.2)	–	–	1.8 (0.6)	TR	4.5 (2.8)	–	13.2 (0.9)
<i>Pedobacter</i> sp. (Ib2)	3.5	30.1	–	–	1.6	TR	3.8	TR	11.3
<i>Pedobacter</i> sp. (II)	1.6	14.2	–	–	3.0	1.1	2.2	–	12.4
<i>S. faecium</i>	1.5	32.2	1.4	–	2.1	TR	–	–	10.0
<i>S. mizutae</i>	TR	9.5 (2.4)	–	–	TR	TR	3.7 (0.2)	–	22.1 (0.6)
<i>S. multivorum</i>	–	31.6 (1.2)	–	TR	5.3 (1.4)	TR	TR	–	7.1 (2.2)
<i>S. spiritivorum</i>	TR	21.1 (1.5)	–	–	2.7 (0.1)	TR	1.7 (0.3)	–	12.5 (0.8)
<i>S. thalophilum</i>	–	23.2 (0.8)	–	3.2 (0.1)	6.3 (0.3)	–	–	–	10.0 (0.1)
[<i>Fa.</i>] <i>yabuuchiae</i>	TR	19.1	–	–	1.8	TR	2.0	–	13.1
[<i>F.</i>] <i>canadensis</i> †	2.7	ND‡	–	–	2.5	1.1	2.5	–	17.7

* SDS-PAGE or DNA hybridization sub-group numbers are shown in parentheses (see Table 1). All strains in Table 1 were studied.

† Contains also 1.5% 17:1 ω 6c and 2.8% of summed feature 5 (iso-17:1 I or anteiso-17:1 B, or both).

‡ The fatty acids iso-15:0(2-OH) and 16:1 ω 7c were not separated using the MIS system. A peak counting for 14.3% of the total fatty acid content eluted with that retention time. The relative amounts of both FAMES were not determined.

culture collection. Since the species is psychrotrophic and differs in many physiological characteristics (24), it can be considered as not belonging to the newly proposed *Pedobacter* taxa and may be distantly related to other *Sphingobacterium* species (28).

Candidatus comitans, a bacterium living in co-culture with the myxobacterium *Chondromyces crocatus* was recently described as a possible new *Sphingobacterium* species (12). Its unique ecological niche and its virtual inability to grow as a pure culture are acceptable grounds to postulate that this bacterium is different from the proposed *Pedobacter* species.

[*Flexibacter*] *canadensis*, a misclassified *Flexibacter* species containing only one strain, is located at the borderline of the *Sphingobacterium* and *Pedobacter* branches. Recovery of more isolates and a further study will be decisive for the creation of a new genus for this species. rRNA sequence analyses revealed an analogous phylogenetic relatedness between *P. heparinus*, the genus *Sphingobacterium* and [*Flexibacter*] *canadensis* (7, 8, 16, 17, 18). Phylogenetic trees from the latter studies and from this study demonstrate that *P. heparinus* is distantly related to [*Flexibacter*] *canadensis* and to the *Sphingobacterium* species.

Although many common biochemical and chemotaxonomical features are found for the genera *Sphingobacterium* and *Pedobacter*, a few of them differentiate each genera and several more can be used for species differentiation (18; Tables 4, 5 and 6). *Pedobacter* can be differentiated from *Sphingobacterium* by the ability to produce heparinase (except *P. piscium*), absence of urease activity, inability of most strains to produce acid from melibiose and the inability to assimilate D-melezitose. Furthermore, all heparinase-producing strains (not *P. piscium*) do not show trypsin activity and only *Sphingobacterium* species, except *S. mizutae*, demonstrate α -fucosidase activity. *Sphingobacterium* species are also differentiated by greater amounts of iso-15:0(2-OH) and *Pedobacter* taxa, except *P. saltans*, possess significantly higher amounts of 16:1 ω 5c. We conclude that when considering the differences in $T_{m(e)}$ values of DNA-rRNA hybridizations as well as the above-mentioned phenotypical differences, there are sufficient grounds for the description of a new genus, *Pedobacter*. Thus far, all heparinase-producing bacteria were isolated from soil or activated sludge, whereas most *Sphingobacterium* strains are of clinical origin.

Proposal of new species

As reported before (26, 27), the heparinase-producing bacteria were classified by SDS-PAGE, DNA-rRNA hybridization, G+C content and phenotypical analysis into three main clusters. Cluster I was further subdivided into two sub-groups Ia and Ib. Table 3 demonstrates that these sub-groups contain respectively three (Ia1, Ia2 and Ia3) and two (Ib1 and Ib2) DNA hybridization groups. DNA hybridization group Ia1, encompassing solely the type strain of *S. heparinum*, is reclassified as *P. heparinus*. Six new isolates of DNA hybridization group Ia3 are classified as a new species named *P. africanus*. Because the other taxa, Ia2, Ib1 and Ib2, do not contain a set of independently isolated strains no new species names are proposed for these DNA hybridization groups. They can be considered as yet-unnamed genospecies of the genus *Pedobacter*. Isolation of more strains may lead to the description of new species names for these sub-groups. Cluster II is shown to be more related to *P. heparinus* [$T_{m(e)} = 74.2^\circ\text{C}$] than to *P. saltans* (cluster III) [$T_{m(e)} = 71.6^\circ\text{C}$]. Due to the presence of a single isolate in cluster II, the strain is not named and is described as *Pedobacter* sp. For cluster III, a very homogeneous (DNA hybridization levels of 94% and higher) and separate DNA hybridization group, the species name *P. saltans* is proposed.

Although all species and genospecies are phenotypically highly similar, they can be differentiated by a combination of tests (Tables 5 and 6). Also, Steyn *et al.* (27) performed a cluster analysis of carbon assimilation tests and found a comparable clustering of the DNA hybridization sub-groups. Only the single strain (LMG 10342) of sub-group Ib2 grouped among strains

of *P. africanus* (sub-group Ia3). Data presented in Table 5 demonstrate that some phenotypic features differentiate the latter two taxa. *P. heparinus* can be differentiated from most other taxa of the genus by its ability to grow on sorbitol and L-fucose and its lack of valine arylamidase. *P. africanus* can be discriminated by its ability to assimilate several amino acids (Table 5). *P. piscium* is the only *Pedobacter* species containing trypsin. *P. saltans* can phenotypically be differentiated from most *Pedobacter* strains by its inability to assimilate D-cellobiose and the ability to utilize glycerol. Most *P. saltans* strains have a peculiar gliding, dancing motility. Strain LMG 10351 (cluster II) is metabolically more inert. Only six of 98 compounds of API 50CH and 50AO are assimilated, whereas several amino acids (API 50AA) are utilized. Within *Pedobacter*, only the fatty acid composition of strain LMG 10351 (cluster II) is clearly different from the other species, having a greater amount of iso-15:0 and less 16:1 ω 7c. Small differences in the amounts of 15:1 ω 6c and 16:0(3-OH) are demonstrated for *P. saltans* (Table 3). *P. piscium* contains a measurable amount of anteiso-17:1 ω 9c. Phenotypic differences among species of the genus *Sphingobacterium* are presented in Table 5 and summarized by Takeuchi & Yokota (28). *S. mizutae* and *S. thalpophilum* are characterized by different amounts of the fatty acids 16:1 ω 7c and 16:0(2-OH), respectively.

Proposal of *Sphingobacteriaceae* fam. nov.

Gherna & Woese (8) classified *C. heparina*, *Sphingobacterium* species and [*Flexibacter*] *canadensis* in the 'Sphingobacter' rRNA sub-group of the 'Flavobacter-Bacteroides' phylum. DNA-rRNA hybridization data demonstrated an analogous separate and homogeneous position of these taxa within superfamily V (*Cytophaga-Flavobacterium-Bacteroides* group) (1, 23; Fig. 1). Differences in $T_{m(e)}$ values of up to 12 °C are found within bacterial families (6, 29). Based on a similar range in $T_{m(e)}$ of 13 °C as well as other common features, we propose here the bacterial family *Sphingobacteriaceae*. Taxa within the family are the genera *Sphingobacterium* and *Pedobacter*, [*Flexibacter*] *canadensis* and *Candidatus* comitans. Differentiating features from other 'flavobacteria', e.g. the emended family *Flavobacteriaceae* (1), are the possession of sphingolipids, the presence of the MK-7 quinone system, a higher mean G+C content and a unique fatty acid content (iso-15:0, iso-15:0(2-OH), iso-15:0(3-OH), 16:0, 16:1 ω 7c, 16:0(3-OH) and iso-17:0(3-OH) as the most important components).

Description of *Pedobacter* gen. nov.

Pedobacter (Pe'do.bac.ter. Gr. n. *pedon* soil; M.L. n. *bacter* from the Gr. n. *baktron* rod or staff; M.L. masc. n. *Pedobacter* rod or staff from soil).

The description below is based on data from this study, as well as on previous descriptions. Most strains are

Table 5. Differentiating biochemical features of the *Pedobacter* and *Sphingobacterium* taxa studied

For the taxa, the name, SDS-PAGE or DNA hybridization sub-group number, and LMG number or number of strains investigated (in parentheses) is given. +, Positive; -, negative; v, variable; the number of strains positive for the respective feature is shown in parentheses; ND, not determined. All strains are positive for the following features: aerobic growth, production of a yellow or creamy white pigment on nutrient agar, aesculin hydrolysis, presence of catalase, oxidase, acid and alkaline phosphatases, esterase lipase (C8), leucine arylamidase, α -glucosidase, β -galactosidase and *N*-acetyl- β -glucosaminidase; assimilation of (API 150) D-glucose, D-mannose, amygdalin and glucosamine. Additionally, all *Pedobacter* strains (*P. piscium* and *Sphingobacterium* strains not tested) contain lysine decarboxylase, phosphoamidase; they produce acetoin from sodium pyruvate and degrade chondroitin sulfate. All strains are negative for the following features: Gram stain, motility, sporulation, indole production, acid production from inositol and adonitol; assimilation of (API 150) erythritol, L-xylose, methyl xyloside, L-sorbose, dulcitol, inositol, xylitol, D-tagatose, D-fucose, L-arabitol, gluconate, acetate, propionate, butyrate, iso-butyrate, n-valerate, iso-valerate, n-vaproate, heptanoate, caprylate, pelargonate, caprate, oxalate, malonate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, glycolate, DL-lactate, DL-glycerate, DL-3-hydroxybutyrate, D-malate, D-tartrate, L-tartrate, meso-tartrate, laevulinate, 2-oxoglutarate, citraconate, itaconate, mesaconate, aconitate, phenylacetate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, iso-phthalate, terephthalate, glycine, D- α -alanine, L- α -alanine, L-leucine, L-isoleucine, L-norleucine, L-valine, DL-norvaline, DL-2-aminobutyrate, L-cysteine, L-methionine, L-phenylalanine, D-tryptophan, L-tryptophan, trigonelline, L-citrulline, DL-kynurenine, betaine, β -alanine, DL-3-aminobutyrate, DL-4-aminobutyrate, DL-5-aminobutyrate, 3-aminobenzoate, 4-aminobenzoate, urea, acetamide, sarcosine, ethylamine, butylamine, amylamine, ethanolamine, benzylamine, diaminobutane, spermine, histamine and tryptamine. Additionally, all *Pedobacter* strains (*P. piscium* and *Sphingobacterium* strains not tested) do not contain lysine deaminase, arginine dihydrolase, phenylalanine deaminase and tryptophan deaminase; they do not produce H₂S from thiosulfate and do not grow on MacConkey agar.

Feature	Taxon													
	<i>P. heparinus</i> 1a1 (2)	<i>P. africanus</i> 1a3 (6)	<i>P. piscium</i> (2)	<i>P. saltans</i> III (4)	<i>Pedobacter</i> sp. 1a2 (2)	<i>Pedobacter</i> sp. 1b1 (2)	<i>Pedobacter</i> sp. 1b2 LMG 10342	<i>Pedobacter</i> sp. II LMG 10351	<i>S. spiritivorum</i> (2)	<i>S. multivorum</i> (2)	<i>S. mizutae</i> (2)	<i>S. thalpoophilum</i> (2)	<i>S. faecium</i> (1)	
Growth at 37 °C	−, +*	v(1)	−*	v(3)	−	−	−	+	+	+	+	+	+	
Nitrate reduction	−, −*	−	−*	−	−	−	−	−	−*	−*	−*	−*	−*	
Gelatin hydrolysis	−, −*	v(1)	−*	−	−	−	−	ND	−*	v*	−*	−*	−*	
Urease	−, −*	−	−*	−	−	−	−	−	+	+	+	+	+	
Heparinase	+	+	−	+	+	+	+	+	−	−	−	−	−	
DNase	−, +*	−	−	−	−	−	−	−	+	+	+	−*	+	
Acid production from:														
Sucrose	−, +*	−	+	−	−	−	−	−	+	+	+	+	+	
Sorbitol	−, +*	−	−*	−	−	−	−	−	−*	−*	−*	−*	−*	
Melibiose	−, −*	−	v*	−	−	−	+	−	+	+	+	+	+	
Mannitol	−, −*	−	v*	−	−	−	−	−	+	−	−	−	−	
Glucose	v, +*	v(1)	+	v(2)	v	v	−	−	+	+	+	+	+	
Lactose	−, +*	−	+	−	−	v	−	−	+	+	+	+	+	
L-Arabinose	v, +*	v(2)	v*	v(3)	v	v	−	−	−*	+	+	+	+	
Rhamnose	−, −*	−	−*	−	−	−	−	−	−*	v*	−*	+	−*	
Enzyme activities (API ZYM):														
Esterase	−, +*	−	+	−	−	−	−	−	+	+	+	+	+	
Lipase (C14)	−, −*	−	v*−	−	−	−	−	−	+	v*	+	−*	v*	
Valine arylamidase	−, −*	v(4)	+	v(1)	v	+	+	+	+	+	+	+	+	
Cystine arylamidase	−, +*	−	+	−	−	−	−	−	+	+	+	+	+	
Trypsin	−, −*	−	+	−	−	−	−	−	+	+	+	+	−*	
Chymotrypsin	−, +*	−	+	−	−	−	−	−	+	+	+	+	+	
α-Galactosidase	−, +*	−	+	−	−	−	−	−	+	+	−*	+	+	
β-Glucuronidase	−, −*	−	v*	v(1)	−	−	−	−	+	v*	−*	+	v*	
β-Glucosidase	−, +*	v(2)	+	+	v	+	+	−	+	+	+	+	+	
α-Mannosidase	−, +*	v(1)	+	−	−	−	+	−	+	+	+	+	+	
α-Fucosidase	−, −*	−	−*	−	−	−	−	−	+	+	−*	+	v*	
Assimilation of (API 50CH, 50AO, 50 AA):														
Glycerol	−	−	−	+	−	v	+	−	v	−	−	+	+	
D-Arabinose	−	v(2)	−	v(3)	v	+	+	−	−	−	+	+	−	
L-Arabinose	+	v(4)	+	+	v	+	+	−	−	+	v	+	+	
Ribose	−	v(1)	−	−	+	−	−	−	−	−	−	−	−	
D-Xylose	+	v(4)	+	+	+	+	+	−	+	+	+	+	−	
Adonitol	+	−	−	+	+	+	−	−	−	−	−	+	−	
D-Galactose	+	+	v	+	+	v	+	−	v	+	+	+	+	
D-Fructose	+	v(2)	+	v(3)	+	+	−	+	+	+	+	+	+	
Rhamnose	+	+	v	+	+	+	+	−	+	+	−	+	+	
Mannitol	+	−	−	−	+	+	−	−	+	−	−	−	−	
Sorbitol	+	−	−	−	+	v	−	−	−	−	−	−	−	
Methyl D-mannoside	+	+	−	−	−	+	+	−	+	+	+	+	+	
Methyl D-glucoside	+	+	+	v	+	+	+	+	+	+	+	+	+	
Arbutin	v	v(3)	+	+	v	+	+	+	+	+	+	+	+	
Salicin	+	v(4)	+	+	+	+	+	−	+	+	+	+	+	
D-Cellobiose	+	+	+	−	+	+	+	−	+	+	+	+	+	

Table 5 (cont.)

Feature	Taxon												
	<i>P. heparinus</i> 1a1 (2)	<i>P. africanus</i> 1a3 (6)	<i>P. piscium</i> (2)	<i>P. saltans</i> III (4)	<i>Pedobacter</i> sp. 1a2 (2)	<i>Pedobacter</i> sp. 1b1 (2)	<i>Pedobacter</i> sp. 1b2 LMG 10342	<i>Pedobacter</i> sp. 11 LMG 10351	<i>S. spiritivorum</i> (2)	<i>S. multivorum</i> (2)	<i>S. mizutae</i> (2)	<i>S. thalophilum</i> (2)	<i>S. faecium</i> (1)
Maltose	+	v(5)	+	+	+	+	+	—	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	—	+	+	+	+	+
D-Melibiose	+	+	+	v(3)	+	+	+	—	+	+	+	+	+
Sucrose	+	+	+	v(3)	+	+	+	—	+	+	+	+	+
Trehalose	+	+	+	v(2)	+	+	+	—	+	+	+	+	+
Inulin	—	—	—	—	—	—	—	—	v	v	—	+	+
D-Melezitose	—	—	—	—	—	—	—	—	+	+	+	+	+
D-Raffinose	—	—	+	v(1)	—	+	—	—	+	+	+	+	+
Starch	—	v(1)	+	—	—	+	+	+	+	+	+	+	+
Glycogen	—	—	—	—	—	v	—	—	—	v	—	—	—
β -Gentobiose	+	v(5)	+	+	+	+	+	—	+	+	+	+	+
D-Turanose	+	+	+	v(3)	+	+	+	—	+	+	+	+	+
D-Lyxose	—	—	—	—	—	—	—	—	—	—	+	—	—
L-Fucose	+	v(2)	—	—	v	v	—	—	—	—	—	—	—
D-Arabitol	—	—	—	—	v	—	—	—	+	—	—	—	—
2-Keto-gluconate	—	—	—	—	—	v	—	—	—	—	—	—	—
5-Keto-gluconate	—	—	—	—	—	—	+	—	—	—	—	—	—
Succinate	—	v(1)	—	—	—	—	—	—	—	v	—	—	—
Fumarate	—	—	—	—	—	—	—	—	—	v	—	—	—
L-Malate	—	—	—	—	—	—	—	—	—	v	—	—	—
Pyruvate	+	v(2)	—	—	+	v	+	—	—	—	v	—	—
Citrate	—	—	—	—	—	—	—	—	—	—	v	—	—
L- α -Alanine	—	v(1)	—	—	—	—	—	—	—	—	—	—	—
L-Serine	v	+	v	—	—	—	—	—	—	—	v	—	—
L-Threonine	v	+	v	—	+	—	—	—	—	v	—	—	—
L-Tyrosine	—	+	—	—	—	—	—	+	—	v	+	—	—
L-Histidine	—	v	—	—	—	—	—	+	—	v	+	—	—
L-Aspartate	—	v	—	—	—	—	—	+	—	v	—	—	—
L-Glutamate	—	+	+	—	+	+	+	+	—	+	+	—	+
L-Ornithine	—	v(1)	—	—	—	—	—	+	—	—	+	—	—
L-Lysine	—	—	—	—	—	—	—	—	—	—	v	—	—
L-Arginine	—	v(1)	—	—	—	—	—	—	—	—	v	—	—
L-Proline	—	v(1)	+	—	—	—	—	+	—	—	+	—	—
Creatine	—	v(1)	—	—	—	—	—	—	—	—	—	—	—
G + C (mol %)	42.3–43.0	43.7–44.2	40.4–41.0	36.9–37.1	42.3–42.9	37.4–37.5	39.5	41.2	39.8	39.9–40.5	39.3–40	44–44.2	37.3

* Data from Takeuchi & Yokota (28).

heparinase-producing, obligately aerobic, Gram-negative rods, varying in form and length from 0.7 to 6 μ m and about 0.5 μ m wide with rounded or slightly tapering ends. No flagella or pili have been observed. Gliding motility has been observed in strains of some species. The colour of the colonies on modified Trypticase soy agar (TSA) and on nutrient agar varies from dirty yellow to creamy white. Pigments do not give the typical flexirubin reaction with 20% KOH. Sizes of the colonies vary from 1 to 5 mm in diameter on both media indicated above. On modified TSA, colonies are round, convex or slightly umbonate with entire margins. On nutrient agar, colonies are round to slightly irregular and spreading, convex to umbonate to flat with entire to scalloped margins. All members (except *P. piscium*) grow on heparin, which is degraded by inducible enzymes. Good growth occurs on nutrient agar or regular and modified TSA, but random isolates may inexplicably lose viability. All or most strains contain cytochrome-c oxidase, catalase, acid and alkaline phosphatases, esterase lipase (C8), leucine-arylamidase, α -glucosidase, β -galactosidase, *N*-acetyl

β -glucosaminidase, lysine decarboxylase and phosphoamidase. They produce acetoin from pyruvate, degrade chondroitin sulfate, hydrolyse aesculin, and assimilate D-glucose, D-mannose, amygdalin and glucosamine. None or almost none of the strains produce H_2S from thiosulfate and indole from tryptophan. Urease, lipase, β -glucuronidase, α -fucosidase, lysine deaminase, arginine dihydrolase, phenylalanine deaminase and tryptophan deaminase are absent. No reduction of nitrate, growth on MacConkey agar, or gelatin liquefaction occurs. No assimilation or acid production is detected for a large number of substrates tested (Table 5). Particular taxa assimilate or produce acid from some carbohydrates, which can be used as differentiating features (Table 5). Organic acids, except pyruvate, and a few amino acids and amines are utilized by only some strains. Sensitivity to antibiotics is described in Table 6.

All the strains contain iso-15:0, iso-15:0(2-OH), iso-15:0(3-OH), 16:0, 16:1 ω 5c, 16:1 ω 7c, 16:0(3-OH), iso-17:0(3-OH) and iso-17:1 ω 9c as major cellular

Table 6. Sensitivity to antibiotics of the *Pedobacter* taxa (except *P. piscium*) studied

All strains studied are sensitive to sulfamethoxazole (128 µg) and cotrimoxazole (160 µg) and are resistant to kanamycin (2 µg), amikacin (16 µg), tobramycin (4 µg) and sisomicin (10 µg). For the taxa, the name, SDS-PAGE or DNA homology sub-group number, and LMG number or number of strains investigated (in parentheses) is shown. +, Positive; –, negative; v, variable; the number of strains positive for the respective feature are shown in parentheses.

Susceptibility (µg)	Taxon						
	<i>P. heparinus</i> Ia1 (2)	<i>P. africanus</i> Ia3 (6)	<i>P. saltans</i> III (4)	<i>Pedobacter</i> sp. Ia2 (2)	<i>Pedobacter</i> sp. Ib1 (2)	<i>Pedobacter</i> sp. Ib2 LMG 10342	<i>Pedobacter</i> sp. II LMG 10351
Ampicillin (8)	–	–	v(3)	–	–	–	+
Chloramphenicol (8)	+	v(1)	+	–	–	+	+
Cephalothin (16)	+	v(2)	–	–	–	–	+
Penicillin (0.03)	+	v(3)	v(1)	–	–	–	–
Carbenicillin (128)	+	–	+	–	+	+	+
Tetracycline (2)	+	–	+	–	–	+	+
Gentamicin (4)	–	–	v(1)	–	–	–	–
Cephmandole (16)	+	–	v(3)	–	v	+	+
Methicillin (4)	+	v(5)	v(3)	–	–	+	–
Erythromycin (4)	+	–	v(3)	–	+	+	+
Cephalexin (16)	+	v(2)	v(1)	–	+	+	+
Cefoxitin (10)	+	v(1)	–	–	v	+	–

Fig. 1. rRNA cistron similarity dendrogram representing the family *Sphingobacteriaceae* as part of rRNA superfamily V. SDS-PAGE or DNA hybridization sub-group numbers are shown in parentheses for unnamed *Pedobacter* species. Abbreviations: Fa., *Flavobacterium*; F., *Flexibacter*; P., *Pedobacter*; S., *Sphingobacterium*. The results are expressed as $T_m(e)$ (°C) versus the reference rRNAs (see Table 2).

fatty acids. All strains tested contain sphingolipids and the MK-7 menaquinone system. The G + C content is 36–45 mol% (T_m). Temperature range is normally between 5 and 30 °C but some strains may grow at 37 °C. The habitat is soil, activated sludge, or fish. The type species is *Pedobacter heparinus*.

Description of *Pedobacter heparinus* (Payza and Korn 1956; emend. Christensen 1980) comb. nov.

Pedobacter heparinus (he.pa.ri'nus. Gr. n. *hepar* liver; M.L. masc. adj. *heparinus* of or pertaining to degradation of heparin, acidic mucoheteropolysaccharide with sulfate groups from various animal tissues).

Cells vary from short rods of 0.7 µm to relatively long rods of 6 µm and 0.5 µm wide with rounded or slightly tapered ends. Protrusions can be observed on the cell surface. Gliding motility has been observed. Colour of colonies varies from translucent yellowish to dirty yellow on modified TSA, and from translucent yellow to bright yellow on nutrient agar. Colonies on modified TSA are 1–4 mm in diameter, round, convex or slightly umbonate with entire margins and convex with entire margins. On nutrient agar, colonies are round to spreading, convex to umbonate to flat with entire to scalloped margins.

General characteristics are those as given for the genus. The organism can be differentiated from most other taxa of the genus by its ability to grow on sorbitol and L-fucose, and the absence of valine arylamidase. Additional differentiating features are given in Tables 4, 5 and 6. Some other characteristics are described by Christensen (2) and Takeuchi & Yokota (28). The G + C content is 42–43 mol% (T_m); the genome size of the type strain LMG 10339 is 2.23×10^9 Da (21). The organism is isolated from soil. Type strain is LMG 10339 (= ATCC 13125).

Description of *Pedobacter africanus* gen. nov., sp. nov.

Pedobacter africanus (a.fri.ca'nus. M.L. masc. adj. *africa* from Africa).

Colonies on modified TSA are 2–5 mm in diameter, translucent, yellow, smooth, round, convex to slightly umbonate with entire margins. On nutrient agar colonies are 2–4 mm in diameter, translucent, yellow, smooth, round, convex, with entire to slightly scalloped margins. Cell morphology is as described for the genus. No gliding motility has been observed. General features are those as given for the genus. Strains of the species can be discriminated by their ability to assimilate several amino acids (Table 5). Additional characteristics are given in Tables 4, 5 and 6. The G + C content is 43–45 mol%. Strains are isolated from soil and activated sludge. Type strain is LMG 10353.

Description of *Pedobacter piscium* (Takeuchi and Yokota 1992) comb. nov.

Pedobacter piscium (pis'ci.um. L. n. *piscis* a fish; L. gen. pl. *piscium* of fishes).

Colonies on modified TSA are 2–5 mm in diameter, translucent, yellow, smooth, round, low convex with entire margins. On nutrient agar, colonies are 2–4 mm in diameter, opaque, smooth, round, convex, with entire margins. A yellow or creamy white non-fluorescent pigment is produced. Cell size is 0.5 µm wide and 0.5–1 µm long. Cell morphology is as described for the genus. No gliding has been observed. Features are as given for the genus. *P. piscium* is the only *Pedobacter* species producing trypsin. Characteristic for its fatty acid content is a measurable amount of anteiso-17:1ω9c. More detailed characteristics are given in Tables 4 and 5 and by Takeuchi & Yokota (28). The growth temperature ranges between 5 and 30 °C, no growth occurs at 37 °C. The G + C content is 40–43 mol%. Strains are probably isolated from fish. Type strain is LMG 14023 (= ATCC 13125, IFO 12017).

Description of *Pedobacter saltans* sp. nov.

Pedobacter saltans (sal'tans. L. v. *saltare* to dance; *saltans* L. adj. dancing, referring to its peculiar dancing or gliding motility).

Colonies on modified TSA are smooth, light yellow to yellow, translucent, round, 2–5 mm in diameter, convex to slightly umbonate with entire margins. On nutrient agar colonies are smooth, yellow, round, 2–4 mm in diameter, convex with entire to scalloped margins. Three out of four isolates exhibit a peculiar gliding motility without any special manipulations. Features are those as given for the genus. *P. saltans* can phenotypically be differentiated from most *Pedobacter* strains by its inability to assimilate D-cellobiose and the ability to utilize glycerol. Additional characteristics are given in Tables 4, 5 and 6. The G + C content is 36–38 mol%. Strains are isolated from soil. Type strain is LMG 10337.

Description of *Sphingobacteriaceae* fam. nov.

Descriptive information for the family is taken from the literature (1, 3, 5, 9, 12, 17, 28, 32) and the present paper. Organisms belonging to the family are Gram-negative. Cells are short to relatively long rods and are usually 0.3–0.6 µm wide and 0.5–6 µm long (except [*Flexibacter*] *canadensis* which is filamentous) and are non-sporulating. Flagella are absent. Non-motile or motile by gliding. Aerobic growth with optimum temperature in a range of 10–30 °C ([*Flexibacter*] *canadensis* also grows as a facultative anaerobe). Colonies are usually yellow pigmented. Chemoorgano-

trophic. All members produce catalase, oxidase and phosphatase, are indole-negative, and can utilize a large number of carbohydrates. Few organic acids and amino acids are utilized. Dominant fatty acids are iso-15:0, iso-15:0(2-OH), iso-15:0(3-OH), 16:0, 16:1 ω 7c, 16:0(3-OH) and iso-17:0(3-OH). Menaquinone 7 is the major respiratory quinone. Presence of sphingolipids. Homospermidine is the major polyamine. The G+C content is 36–45 mol%. Members of this family are commonly isolated from soil or activated sludge and from clinical specimens. No species are considered as true pathogens.

The type genus is *Sphingobacterium*. Other members are *Pedobacter* and [*Flexibacter*] *canadensis*. All these organisms belong to the same rRNA branch within rRNA superfamily V (the *Cytophaga*–*Flavobacterium*–*Bacteroides* group). Differential characteristics between the taxa belonging to the family *Sphingobacteriaceae* are given in Tables 4, 5 and 6 and the literature (3, 12, 24, 28, 32). The family *Sphingobacteriaceae* belongs to the order *Cytophagales* Leadbetter 1974, 99^{AL} (22).

ACKNOWLEDGEMENTS

K.K. is indebted to the Fund for Medical Scientific Research, Belgium, for research and personnel grants. Part of the research was performed in the framework of the CEC contract BIO2-CT94-3098 and also co-financed by the Prime Minister's Services – Federal Office for Scientific, Technical and Cultural Affairs, Belgium.

REFERENCES

- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.
- Christensen, P. (1980a). Description and taxonomic status of *Cytophaga heparina* (Payza and Korn) comb. nov. (basonym: *Flavobacterium heparinum* Payza and Korn 1956). *Int J Syst Bacteriol* **30**, 473–475.
- Christensen, P. (1980b). *Flexibacter canadensis* sp. nov. *Int J Syst Bacteriol* **30**, 429–432.
- Cowan, S. T. (1979). *Cowan & Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.
- Dees, S. B., Carlone, G. M., Hollis, D. & Moss, C. W. (1985). Chemical and phenotypic characteristics of *Flavobacterium thalophilum* compared with those of other *Flavobacterium* and *Sphingobacterium* species. *Int J Syst Bacteriol* **35**, 16–22.
- De Ley, J. (1992). The *Proteobacteria*: ribosomal RNA cistron similarities and bacterial taxonomy. In *The Prokaryotes*, 2nd edn, vol. 2, pp. 2111–2140. A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.
- Dobson, S. J., Colwell, R. R., McMeekin, T. A. & Franzman, P. D. (1993). Direct sequencing of the polymerase chain reaction-amplified 16S rRNA gene of *Flavobacterium gondwanense* sp. nov. and *Flavobacterium salegens* sp. nov., two new species from a hypersaline antarctic lake. *Int J Syst Bacteriol* **43**, 77–83.
- Gherna, R. & Woese, C. R. (1992). A partial phylogenetic analysis of the 'Flavobacter-Bacteroides' phylum: basis for taxonomic restructuring. *Syst Appl Microbiol* **15**, 513–51.
- Hamana, K. & Matsuzaki, S. (1991). Polyamine distributions in the *Flavobacterium*–*Cytophaga*–*Sphingobacterium* complex. *Can J Microbiol* **37**, 885–888.
- Holmes, B., Owen, R. J. & Hollis, D. G. (1982). *Flavobacterium spiritivorum*, a new species isolated from human clinical specimens. *Int J Syst Bacteriol* **32**, 157–165.
- Holmes, B., Owen, R. J. & Weaver, R. E. (1981). *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as group IIk, biotype 2. *Int J Syst Bacteriol* **31**, 21–34.
- Jacobi, C. A., Reichenbach, H., Tindall, B. J. & Stackebrandt, E. (1996). *Candidatus comitans*, a bacterium living in coculture with *Chondromyces crocatus* (myxobacteria). *Int J Syst Bacteriol* **46**, 119–122.
- Joubert, J. J., van Rensburg, E. J. & Pitout, M. J. (1984). A plate method for demonstrating the breakdown of heparin and chondroitin sulfate by bacteria. *Microbiol Methods* **2**, 197–202.
- Joubert, J. J. (1985). *Kenmerking van heparinase produserende mikro-organismes*. MD thesis, University of Pretoria.
- Kerstens, K., Hinz, K.-H., Hertle, A., Segers, P., Lievens, A., Siegmann, O. & De Ley, J. (1984). *Bordetella avium* sp. nov. isolated from the respiratory tracts of turkeys and other birds. *Int J Syst Bacteriol* **34**, 56–70.
- Manz, W., Amann, R., Ludwig, W., Vancanneyt, M. & Schleifer, K.-H. (1996). Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* **142**, 1097–1106.
- Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.
- Nakagawa, Y. & Yamasato, K. (1996). Emendation of the genus *Cytophaga* and transfer of *Cytophaga salmonicolor* to *Marinilibilia* gen. nov.: phylogenetic analysis of the *Flavobacterium*–*Cytophaga* complex. *Int J Syst Bacteriol* **46**, 599–603.
- Paster, B. J., Ludwig, W., Weisburg, W. G., Stackebrandt, E., Hespell, R. B., Hahn, C. M., Reichenbach, H., Stetter, K. O. & Woese, C. R. (1985). A phylogenetic grouping of the bacteroides, cytophagas, and certain flavobacteria. *Syst Appl Microbiol* **6**, 34–42.
- Payza, A. N. & Korn, E. D. (1956). The degradation of heparin by bacterial enzymes. 1. Adaptation and lyophilized cells. *J Biol Chem* **223**, 853–864.
- Reichenbach, H. (1989a). Genus I. *Cytophaga* Winogradsky 1929, 577^{AL}. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2020. Baltimore: Williams & Wilkins.
- Reichenbach, H. (1989b). Order I. *Cytophagales* Leadbetter 1974, 99^{AL}. Edited by J. T. Staley, M. P. Bryant, N. Pfennig

- & J. G. Holt. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2011–2013. Baltimore: Williams & Wilkins.
23. Segers, P., Mannheim, W., Vancanneyt, M., De Brandt, K., Hinz, K.-H., Kersters, K. & Vandamme, P. (1993). Description of *Riemerella anatipestifer* gen. nov., comb. nov., the causative agent of septicaemia anserum exsudativa and its phylogenetic affiliation within the *Flavobacterium-Cytophaga* rRNA hybridization group. *Int J Syst Bacteriol* **43**, 768–776.
 24. Shivaji, S., Ray, M. K., Shyamale Rao, N., Saisree, L., Jaganadham, M. V., Seshu Kumar, G., Reddy, G. S. N. & Bhargava, P. M. (1992). *Sphingobacterium antarcticus* sp. nov., a psychotrophic bacterium from soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* **42**, 102–106.
 25. Sonnenwirth, A. C. (1980). Bacteriological methods. In *Gradwohl's Clinical Laboratory Methods and Diagnosis*, vol. 2, 8th edn, pp. 1338–1377. Edited by A. C. Sonnenwirth & W. Jarret. London: C. V. Mosby.
 26. Steyn, P. L., Pot, B., Segers, P., Kersters, K. & Joubert, J. J. (1992). Some novel aerobic heparin-degrading bacteria. *Syst Appl Microbiol* **15**, 137–143.
 27. Steyn, P. L., Joubert, J. J., Segers, P., Pot, B., Vancanneyt, M., Willems, A., Hoste, B. & Kersters, K. (1993). The taxonomic position of some gram-negative aerobic heparinase producing bacteria. In *Advances in the Taxonomy and Significance of Flavobacterium, Cytophaga and Related Bacteria*, pp. 137–151. Edited by P. J. Jooste. Bloemfontein, South Africa: University of Orange Free State Press.
 28. Takeuchi, M. & Yokota, A. (1992). Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalpophilum* comb. nov. and two genospecies of the genus *Sphingobacterium*, and synonymy of *Flavobacterium yabuuchiae* and *Sphingobacterium spiritivorum*. *J Gen Appl Microbiol* **38**, 465–482.
 29. Vandamme, P. & De Ley, J. (1992). Proposal for a new family, *Campylobacteraceae*. *Int J Syst Bacteriol* **41**, 451–455.
 30. Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. & Holmes, B. (1994). New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov. and *Empedobacter* nom. rev. *Int J Syst Bacteriol* **44**, 827–831.
 31. Vannieuwenhuyze, F. & Sandra, P. (1987). Selectivity optimization for the capillary gas chromatographic analysis of bacterial cellular fatty acids. *Chromatographia* **23**, 850–855.
 32. Yabuuchi, E., Kaneko, T., Yano, I., Moss, C. W. & Miyoshi, N. (1983). *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting gram-negative rods in CDC groups IIk-2 and IIb. *Int J Syst Bacteriol* **33**, 580–598.
 33. Yabuuchi, E. & Moss, C. W. (1982). Cellular fatty acid composition of strains of three species of *Sphingobacterium* gen. nov. and *Cytophaga johnsonae*. *FEMS Microbiol Lett* **13**, 87–91.