

## Full Paper

# Distribution of two triamines, spermidine and homospermidine, and an aromatic amine, 2-phenylethylamine, within the phylum Bacteroidetes

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Cellular polyamines of the newly additional 19 species belonging to the class Bacteroides of the phylum Bacteroidetes were analyzed by HPLC to display polyamine distribution as a chemotaxonomic marker within the total 41 species. Three profiles, the presence of spermidine, the presence of homospermidine and the absence of both triamines, corresponded to their phylogenetical positions within the four families of the class. The occurrence of an aromatic amine, 2-phenylethylamine, extracted into cellular polyamine fraction, was also determined within the 121 species distributed within the phylum. This aromatic amine was found in *Cellulophaga lytica*, *Cytophaga latercula*, *Tenacibaculum amylolyticum*, *Tenacibaculum martimum*, *Tenacibaculum mesophilum* and *Psychroflexus torquis* belonging to the family Flavobacteriaceae of the class Flavobacteria, and *Flexibacter flexilis* and *Microscilla marina* belonging to the family Flexibacteraceae of the class Sphingobacteria.

**Key Words**—*Bacteroides*; *Flavobacterium*; homospermidine; phenylethylamine; polyamine; *Porphyromonas*; *Prevotella*; spermidine

## Introduction

The phylum Bacteroidetes (formerly *Cytophaga-Flavobacterium-Bacteroides* phylum) comprise the three classes Bacteroides, Flavobacteria and Sphingobacteria (Bernardet et al., 2002; Boone and Castenholz, 2001; Hofstad et al., 2000; NCBI Home page, 2004; Paster et al., 1994; Weller et al., 2000). Cellular polyamine distribution profile within this phylum, as a phenotypic chemotaxonomic marker, has been studied (Hamana and Matsuzaki, 1990, 1991; Hamana et al., 1995; Hamana and Nakagawa, 2001a, b). Homospermidine was ubiquitously found as the major polyamine in all 16 genera belonging to the family Flavobacteriaceae of the class Flavobacteria and the family Sphin-

gobacteriaceae of the class Sphingobacteria. Fifteen other genera, phylogenetically spread beyond the two families, contained homospermidine or spermidine, or lacked polyamines (Hamana and Nakagawa, 2001a, b; Hosoya and Hamana, 2003).

The class Bacteroides (order Bacteroidales) containing 13 genera is phylogenetically divided into the four families *Prevotellaceae*, *Porphyromonadaceae*, *Bacteroidaceae* and *Rikenellaceae* (Boone and Castenholz, 2001; Miyamoto and Itoh, 2000; Nakagawa and Yamasato, 1996; NCBI Home page, 2004; Paster et al., 1994; Suzuki et al., 1999; Weller et al., 2000). Cellular polyamines of 19 additional species were newly analyzed in the present study to display complete polyamine distribution profiles within the total 41 species (47 strains) of the class Bacteroides.

A recent study of ours first showed the occurrence of an aromatic amine, 2-phenylethylamine ( $\beta$ -phenethylamine), in the cellular polyamine fraction of psychrophilic *Psychroflexus torquis* belonging to the

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family *Flavobacteriaceae* (Hamana and Niitsu, 2001). Therefore, distribution of this aromatic amine was newly analyzed within the 71 species (111 strains), except for the species located in the class Bacteroides, in which 2-phenylethylamine had not been determined (Hamana and Nakagawa, 2001a, b). Furthermore polyamines of 6 newly validated species (7 strains) belonging to the two new genera *Zobellia* (Barbeyron et al., 2001) and *Tenacibaculum* (Suzuki et al., 2001) and new three psychrophilic *Flavobacterium* species (Humphry et al., 2001; Tamaki et al., 2003; Zhu et al., 2003) of the family *Flavobacteriaceae*, and a species of the genus *Dyadobacter* (Chelius and Triplett, 2000) of the family *Flexibacteraceae* (Nakagawa et al., 2002) were analyzed.

### Materials and Methods

Organisms belonging to the class Bacteroides were cultured anaerobically at optimum growth temperature (30–37°C) in GAM broth (Nissui Pharmaceutical Co., Tokyo, Japan) (Hamana et al., 1995). Other flavobacteria were cultivated aerobically at 25°C in marine broth (MB), 199 medium dissolved in seawater (199-SW), tripticase soy broth (TSB) (BBL, MD, USA), and various media listed in IFO, ATCC and IAM (Hamana and Nakagawa, 2001a, b). Three psychrophilic *Flavobacterium* species were grown at 15°C. Cells were harvested in the stationary phase by centrifugation at 3,000×g and washed with 0.8% NaCl and then the pellets were homogenized in equal volumes of cold 1.0 M perchloric acid (PCA) at 4°C. Polyamines extracted into 0.5 M PCA were analyzed by high performance liquid chromatography (HPLC) on an L6000 high speed liquid chromatograph (Hitachi Co., Tokyo, Japan) using a column of cation-exchange resin (Hamana et al., 1995).

### Results and Discussion

Cellular concentrations of polyamines of the 19 strains newly analyzed and the 28 strains previously analyzed (total 47 strains), belonging to 41 species of the class Bacteroides, are shown in Table 1. GAM medium used for their cultivations contained small amounts of putrescine, cadaverine, spermidine and spermine (Table 1). Incorporation of polyamines into the cells from the medium was not excluded. The higher level of 0.40 µmol/g wet weight was defined as

threshold value for synthetic cellular spermidine in the present study.

Twelve *Prevotella* species including six strains newly analyzed, phylogenetically belonging to the family Prevotellaceae, were free of cellular polyamines. Appreciable amounts of spermidine was detected in *Prevotella nigrescens*. *Bacteroides caccae*, *B. stercoris*, *B. uniformis*, *B. vulgatus* and *B. tectus* contained spermidine. Spermidine was absent in *Bacteroides acidifaciens*, *B. fragilis*, *B. ovatus*, *B. pyogens*, *B. suis* and *B. thetaiotaomicron*. Spermidine was found as the major polyamine in the four *Porphyromonas* species, *Bacteroides distasonis*, *Bacteroides merdae*, *Tannerella forsythia* (Maiden et al., 2003; Sakamoto et al., 2002) and *Dysgonomonas gadei* (Hofstad et al., 2000), belonging to the family *Porphyromonadaceae*. Only a trace amount of spermidine was detected in *Porphyromonas levii* (Hamana et al., 1995). Spermidine was the major polyamine in *Bacteroides splanchnicus* and *Bacteroides putredinis* and *Cytophaga xylanolytica*, *Marinilabilia salmonicolor* (Nakagawa and Yamasato, 1996; Suzuki et al., 1999) and *Cytophaga fermentans* contained spermidine as well.

Phylogenetic analyses showed that *Prevotella heparinolytica* and *Prevotella zoogloiformans* were located in the family *Bacteroidaceae* (Paster et al. 1994; Weller et al., 2000) and the two organisms contained spermidine. This chemotaxonomic datum supports reclassification of them. *Bacteroides distasonis* and *Bacteroides merdae* are phylogenetically located in the family *Porphyromonadaceae* together with *Tannerella* and *Dysgonomonas* species and contained spermidine, supporting reclassification of the two *Bacteroides* species. *Bacteroides splanchnicus*, *Bacteroides putredinis*, *Cytophaga xylanolytica* and *Cytophaga fermentans*, containing spermidine, should be reclassified as new genera of the family Rikenellaceae. However authentic *Cytophaga* species of the family *Flexibacteraceae* of the class Sphingobacteria ubiquitously contained homospermidine as the major polyamine (Hamana et al., 1995; Hamana and Nakagawa, 2001b). The occurrence of homospermidine as the major polyamine in *Rikenella microfusus* is a unique polyamine profile within the class Bacteroides (Hamana et al., 1995).

2-Phenylethylamine produced by decarboxylation of L-phenylalanine, was detected as a PCA-extractable cellular amine in psychrophilic *Psychroflexus torquis* growing at 5°C, but not in mesophilic *Psychroflexus*

Table 1. Cellular concentrations of polyamines within the families *Prevotellaceae*, *Bacteroidaceae*, *Porphyromonadaceae* and *Rikenellaceae* of the class Bacteroides.

Organism	Strain number		Polyamines ( $\mu\text{mol/g}$ wet weight):					
			Put	Cad	Spd	HSpd	Spm	Agm
<i>Prevotellaceae</i>								
<i>Prevotella bivia</i>	JCM 6331 <sup>T</sup>	(a)	0.01	0.01	0.13	—	0.02	—
	JCM 6332		—	—	0.08	—	0.02	—
<i>corporis</i>	JCM 8529 <sup>T</sup>	(a)	0.04	—	—	—	—	—
<i>denticola</i>	JCM 8528 <sup>T</sup>	(a)	0.05	—	—	—	—	—
<i>disiens</i>	JCM 6334 <sup>T</sup>	(a)	0.01	0.01	0.01	—	0.01	—
	JCM 6333		—	—	0.10	—	0.02	—
<i>intermedia</i>	JCM 7365 <sup>T</sup>	(a)	0.02	—	0.05	—	0.04	—
<i>loescheii</i>	JCM 8530	(a)	0.06	0.01	0.01	—	—	0.05
<i>melaninogenica</i>	JCM 6325 <sup>T</sup>	(a)	0.02	0.08	0.09	—	0.04	—
	JCM 6321		—	—	—	—	—	—
<i>nigrescens</i>	JCM 6322 <sup>T</sup>		—	—	0.75	—	—	—
<i>oralis</i>	JCM 6330	(a)	0.01	0.01	0.01	—	—	—
<i>oris</i>	JCM 8540 <sup>T</sup>	(a)	0.01	—	0.02	—	—	—
<i>pallens</i>	JCM 11140 <sup>T</sup>		—	—	0.20	—	—	—
<i>ruminicola</i>	JCM 8958 <sup>T</sup>		—	—	0.03	—	—	—
<i>veroralis</i>	JCM 6290 <sup>T</sup>	(a)	0.01	0.01	0.01	—	0.01	—
<i>Bacteroidaceae</i>								
<i>Bacteroides acidifaciens</i>	JCM 10556 <sup>T</sup>		—	—	0.03	—	0.02	—
<i>caccae</i>	JCM 9498 <sup>T</sup>		—	—	0.86	—	0.02	0.15
<i>fragilis</i>	GAI 0675 <sup>T</sup>	(a)	0.01	0.01	0.09	—	0.05	—
	GAI 5560	(a)	0.01	—	0.02	—	0.04	—
<i>ovatus</i>	JCM 5824 <sup>T</sup>	(a)	0.01	0.01	0.03	—	0.02	—
<i>pyogenes</i>	JCM 6294 <sup>T</sup>	(a)	0.01	0.01	0.03	—	0.02	—
<i>stercoris</i>	JCM 9496 <sup>T</sup>		—	—	0.62	—	0.02	—
<i>suis</i>	JCM 6292 <sup>T</sup>	(a)	0.08	0.09	0.08	—	0.04	—
<i>thetaitotaomicron</i>	JCM 5827 <sup>T</sup>	(a)	0.02	—	0.05	—	0.05	—
<i>uniformis</i>	JCM 5828 <sup>T</sup>	(a)	0.08	0.02	0.98	—	0.03	—
<i>vulgatus</i>	JCM 5826 <sup>T</sup>	(a)	0.02	0.02	0.72	—	0.05	0.40
<i>tectus</i>	JCM 10003 <sup>T</sup>		0.01	—	1.10	—	—	—
<i>Prevotella heparinolytica</i> ( <i>Bacteroides heparinolytica</i> )	ATCC 35895 <sup>T</sup>		—	0.08	0.40	—	—	0.08
<i>Prevotella zoogloeiformans</i> ( <i>Bacteroides zoogloeiformans</i> )	ATCC 33285 <sup>T</sup>		—	0.02	1.20	—	—	—
<i>Porphyromonadaceae</i>								
<i>Porphyromonas asaccharolytica</i>	JCM 6326 <sup>T</sup>	(a)	0.04	—	1.14	—	0.03	—
<i>endodontalis</i>	JCM 8526 <sup>T</sup>	(a)	0.05	—	0.50	—	—	0.02
<i>gingivalis</i>	GAI 7802 <sup>T</sup>	(a)	0.01	—	0.95	—	0.01	—
	JCM 8525		—	—	0.82	—	—	—
<i>levii</i>	JCM 8531 <sup>T</sup>	(a)	0.02	0.01	0.02	—	—	—
<i>macacae</i>	ATCC 33141 <sup>T</sup>		—	0.02	0.65	—	—	—
<i>Bacteroides distasonis</i>	JCM 5825 <sup>T</sup>	(a)	0.03	—	0.82	—	0.05	0.20
<i>Bacteroides merdae</i>	JCM 9497 <sup>T</sup>		—	—	0.55	—	—	0.04
<i>Tannerella forsythia</i> ( <i>Bacteroides forsythus</i> )	ATCC 43037 <sup>T</sup>		—	0.10	1.10	—	—	—
<i>Dysgonomonas gadei</i>	ATCC BAA-286 <sup>T</sup>		—	—	0.45	—	—	—
<i>Rikenellaceae</i>								
<i>Bacteroides splanchnicus</i>	ATCC 29572 <sup>T</sup>		—	—	1.05	—	0.02	—
<i>Cytophaga xylanolytica</i>	ATCC 51429 <sup>T</sup>	(b)	—	—	1.07	—	—	—
<i>Marinilabilia salmonicolor</i> biovar <i>agarovorans</i>	IFO 14957 <sup>T</sup>	(a)	0.01	—	0.70	—	—	—
biovar <i>salmonicolor</i>	NCIMB 2216 <sup>T</sup>	(a)	0.05	—	1.25	—	—	—
<i>Cytophaga fermentans</i>	NCIMB 2218 <sup>T</sup>	(a)	—	0.08	2.05	—	—	—
<i>Bacteroides putredinis</i>	ATCC 29800 <sup>T</sup>		—	—	1.40	—	0.02	—
<i>Rikenella microfus</i>	JCM 2053 <sup>T</sup>	(a)	0.04	—	0.02	2.25	0.05	—
GAM medium ( $\mu\text{mol/L}$ )		(a)	0.16	0.10	0.06	—	0.01	—

Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; <sup>T</sup>, type strain; —, not detectable (<0.005); IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, RIKEN (Institute of Chemical and Physical Research), Saitama, Japan; GAI, Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu, Japan; ATCC, American Type Culture Collection, Manassas, Virginia, USA; NCIMB, The National Collections of Industrial, Food and Marine Bacteria, Aberdeen, Scotland, UK. Former names are shown in parentheses. (a), Cited from Hamana et al. (1995); (b), Hamana and Nakagawa (2001b).

Table 2. Cellular concentrations of polyamines within the family *Flavobacteriaceae* of the class *Flavobacteria* and the family *Flexibacteraceae* of the class *Sphingobacteria*.

Organism	Strain number	Medium	Polyamines (μmol/g wet weight)						
			Put	Cad	Spd	HSpd	Spm	Agm	Pea
<i>Flavobacteriaceae</i>									
<i>Cellulophaga lytica</i>	IFO 15985	MB	0.25	—	—	2.10	—	0.35	0.80
	IFO 15986	MB	0.03	—	—	1.75	—	0.25	0.06
	IFO 16020	IFO333	0.95	—	—	2.05	—	0.30	1.02
<i>Cytophaga latercula</i>	IAM 14305 <sup>T</sup>	IAMB68	—	0.95	—	0.54	—	1.50	0.64
<i>Tenacibaculum amylolyticum</i>	IFO 16310 <sup>T</sup>	MB	—	0.50	0.35	1.35	0.20	0.15	0.15
		199-SW	—	0.05	—	1.00	—	0.69	0.90
<i>Tenacibaculum maritimum</i> ( <i>Flexibacter maritimus</i> )	IFO 15991	MB	0.04	0.08	0.01	0.67	—	0.69	0.08
	IFO 15946 <sup>T</sup>	MB	0.04	0.10	0.01	0.59	—	0.30	0.07
<i>Tenacibaculum mesophilum</i>	IFO 16307 <sup>T</sup>	MB	0.06	0.25	0.02	1.05	0.03	0.27	0.04
		199-SW	—	0.05	—	1.40	—	0.45	0.45
<i>Tenacibaculum ovolyticum</i> ( <i>Flexibacter ovolyticus</i> )	IAM 14318 <sup>T</sup>	MB	—	—	—	1.10	—	0.19	—
<i>Zobellia uliginosa</i> ( <i>Cellulophaga uliginosa</i> )	IAM 14312 <sup>T</sup>	199-SW	0.05	—	—	0.90	—	0.15	—
		MB	—	0.10	—	1.20	—	0.30	—
		IAMB69	—	—	—	1.05	—	0.15	—
<i>Flavobacterium frigidarium</i>	NCIMB 13737 <sup>T</sup>	NCIMB475	—	—	—	1.24	—	—	—
		199	—	—	—	0.98	—	—	—
<i>Flavobacterium limicola</i>	JCM 11473 <sup>T</sup>	TSB	0.05	—	—	1.50	—	0.08	—
		199	—	—	—	0.92	—	0.02	—
<i>Flavobacterium omnivorum</i>	JCM 11313 <sup>T</sup>	199	—	—	—	1.02	—	—	—
<i>Flexibacteraceae</i>									
<i>Flexibacter flexilis</i>	IFO 16026	IFO272	—	—	—	1.30	—	0.02	0.02
	IFO 16027	IFO272	—	—	—	1.40	—	0.02	—
<i>Microscilla marina</i>	IFO 16042 <sup>T</sup>	IFO333	0.16	0.50	0.30	—	—	0.02	0.08
<i>Dyadobacter fermentans</i>	ATCC 700827 <sup>T</sup>	ATCC1981	0.02	0.01	0.72	—	0.08	—	—

Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; Pea, 2-phenylethylamine; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; NCIMB, The National Collections of Industrial, Marine and Food Bacteria, Aberdeen, Scotland, UK; ATCC, American Type Culture Collection, Manassas, Virginia, USA; <sup>T</sup>, type strain; —, not detectable (<0.005). Former names are shown in parentheses.

*gondwanensis* growing at 26°C (Hamana and Niitsu, 2001). Among the 111 reanalyzed organisms (71 species) belonging to the phylum Bacteroidetes previously listed (Hamana and Nakagawa, 2001a, b), this aromatic amine was detected in the packed cells of seven organisms, *Cellulophaga lytica*, *Cytophaga latercula*, *Flexibacter flexilis*, three *Tenacibaculum* species and *Microscilla marina* (Table 2). This amine was not found in all of the 47 analyzed strains of the class Bacteroides. As shown in Fig. 1, A–F, this aromatic amine and agmatine were not only detected as a cellular amine but also found in culture supernatant,

indicating extracellular secretion of the two amines, as reported previously (Hamana and Niitsu, 2001). In *Zobellia uliginosa* grown in three different media, agmatine was found only in cellular PCA extract, whereas 2-phenylethylamine was found only in the culture supernatant, as shown in Fig. 1, G–I.

2-Phenylethylamine was not found in the three new psychrophilic *Flavobacterium* species, *F. frigidarium*, *F. limicola* (Tamaki et al., 2003) and *F. omnivorum*, growing at 15°C and containing homospermidine (Table 2). This aromatic amine has not been detected in other psychrophilic flavobacteria, *Flavobacterium hi-*

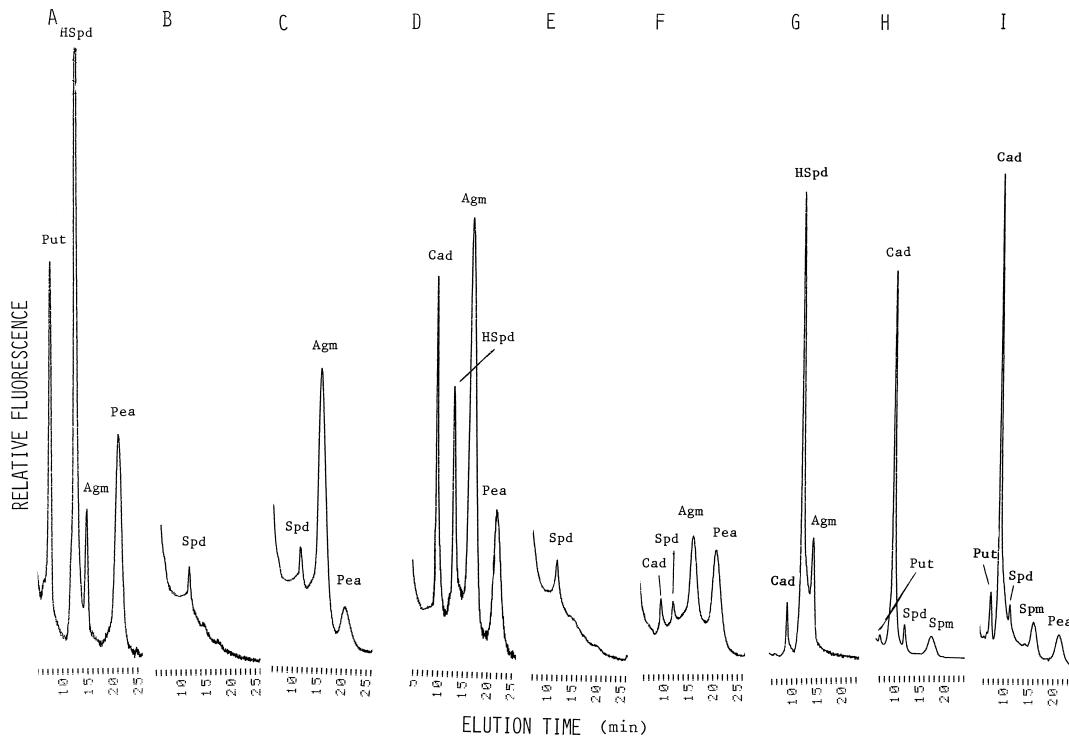


Fig. 1. HPLC analysis of the polyamines extracted from the packed cells of *Cellulophaga lytica* IFO 16020 grown in IFO medium No. 333 (A), from IFO medium No. 333 (B), and from the supernatant after cultivation (C), and the polyamines extracted from the packed cells of *Cytophaga latercula* IAM 14305 grown in IAM medium No. B68 (D), from IAM medium No. B68 (E) and from the supernatant after cultivation (F), and the polyamines extracted from the packed cells of *Zobellia uliginosa* IAM 14312 grown in MB (G), from MB (H) and from the supernatant after cultivation (I).

The abbreviations for polyamines are shown in Tables 1 and 2.

*bernum* (growing at 20°C), *Flavobacterium psychrophilum* (20°C), *Polaribacter filamentum* (4°C), *Polaribacter glomeratus* (15°C), *Psychroserpens burtonensis* (15°C) or *Gelidibacter algens* (10°C) (Hamana and Nakagawa, 2001a, b), indicating that the production of this aromatic amine has no relation to psychrophily. *Dyadobacter fermentans* (Chelius and Triplett, 2000) as well as *Runella slithformis* and *Spirosoma linguale* (Hamana and Nakagawa, 2001a, b), of the family *Flexibacteraceae*, contained spermidine as the major polyamine and lacked 2-phenylethylamine.

Although the determination of net cellular spermidine synthesis was not clear in some strains, the distribution profile of spermidine and homospermidine serves as a phenotypic marker within the phylum Bacteroidetes. Putrescine is produced by decarboxylation of L-ornithine, however, subjected to spermidine and homospermidine syntheses. A part of cadaverine, agmatine and 2-phenylethylamine are secreted into culture medium; their intracellular levels are variable. Therefore cellular levels of putrescine, cadaverine, ag-

matine and 2-phenylethylamine are useful for phenotypic discrimination among species or strains but unsuitable as a chemotaxonomic marker for the phylogenetic classification of the phylum Bacteroidetes.

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#### References

- Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B., and Potin, P. (2001) *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int. J. Syst. Evol. Microbiol.*, **51**, 985–997.
- Bernardet, J.-F., Nakagawa, Y., and Holmes, B. (2002) Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int. J. Syst. Evol. Microbiol.*, **52**, 1049–1070.
- Boone, D. R. and Castenholz, R. W. (2001) Taxonomic outline

- of the Archaea and Bacteria. In *Bergey's Manual of Systematic Bacteriology*, 2nd ed., Vol. 1, ed. by Boone, D. R. and Castenholz, R. W., Springer, Berlin, pp. 155–166.
- Chelius, M. K. and Triplett, E. W. (2000) *Dyadobacter fermentans* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from surface-sterilized *Zea mays* stems. *Int. J. Syst. Evol. Microbiol.*, **50**, 751–758.
- Hamana, K. and Matsuzaki, S. (1990) Occurrence of homospermidine as the major polyamine in the authentic genus *Flavobacterium*. *Can. J. Microbiol.*, **36**, 228–231.
- Hamana, K. and Matsuzaki, S. (1991) Polyamine distribution in the *Flavobacterium-Cytophaga-Sphingobacterium* complex. *Can. J. Microbiol.*, **37**, 885–888.
- Hamana, K., Nakagawa, Y., and Yamasato, K. (1995) Chemotaxonomic significance of polyamine distribution patterns in the *Flavobacterium-Cytophaga* complex and related genera. *Microbios*, **81**, 135–145.
- Hamana, K. and Nakagawa, Y. (2001a) Polyamine distribution profiles in the eighteen genera phylogenetically located within *Flavobacterium-Flexibacter-Cytophaga-Sphingobacterium* complex. *Microbios*, **106**, 7–17.
- Hamana, K. and Nakagawa, Y. (2001b) Polyamine distribution profiles in the newly validated genera and species within the *Flavobacterium-Flexibacter-Cytophaga-Sphingobacterium* complex. *Microbios*, **106** (S-2), 105–116.
- Hamana, K. and Niitsu, M. (2001) Large production of an aromatic amine, 2-phenylethylamine, in a psychrophilic marine bacterium, *Psychroflexus torquis*. *J. Gen. Appl. Microbiol.*, **47**, 103–105.
- Hofstad, T., Olsen, I., Eribe, E. R., Falsen, E., Collins, M. D., and Lawson, P. A. (2000) *Dysgonomonas* gen. nov. to accommodate *Dysgonomonas gadei* sp. nov., an organism isolated from a human gall bladder, and *Dysgonomonas capnocytophagoides* (formerly CDC group DF-3). *Int. J. Syst. Evol. Microbiol.*, **50**, 2189–2195.
- Hosoya, R. and Hamana, K. (2003) Absence of cellular trimines in four novel flavobacteria located in *Flavobacterium-Flexibacter-Cytophaga* complex. *Ann. Gunma Health Sci.*, **24**, 13–16.
- Humphry, D. R., George, A., Black, G. W., and Cummings, S. P. (2001) *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. *Int. J. Syst. Evol. Microbiol.*, **51**, 1235–1243.
- Maiden, M. F. J., Cohee, P., and Tanner, A. C. R. (2003) Proposal to conserve the adjectival form of the specific epithet in the reclassification of *Bacteroides forsythus* Tanner et al. 1986 to the genus *Tannerella* Sakamoto et al. 2002 as *Tannerella forsythia* corrig., gen. nov., comb. nov. Request for an opinion. *Int. J. Syst. Evol. Microbiol.*, **53**, 2111–2112.
- Miyamoto, Y. and Itoh, K. (2000) *Bacteroides acidifaciens* sp. nov., isolated from the caecum of mice. *Int. J. Syst. Evol. Microbiol.*, **50**, 145–148.
- Nakagawa, Y., Sakane, T., Suzuki, M., and Hatano, K. (2002) Phylogenetic structure of the genera *Flexibacter*, *Flexithrix*, and *Microscilla* deduced from 16S rRNA sequence analysis. *J. Gen. Appl. Microbiol.*, **48**, 155–165.
- Nakagawa, Y. and Yamasato, K. (1996) Emendation of the genus *Cytophaga* and transfer of *Cytophaga agarovorans* and *Cytophaga salmonicolor* to *Marinilabilia* gen. nov.: Phylogenetic analysis of the *Flavobacterium-Cytophaga* complex. *Int. J. Syst. Bacteriol.*, **46**, 599–603.
- NCBI (National Center for Biotechnology Information, US National Library of Medicine), Taxonomy browser (Bacteria) (2004) <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/>
- Paster, B. J., Dewhirst, F. E., Olsen, I., and Fraser, G. J. (1994) Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and related bacteria. *J. Bacteriol.*, **176**, 725–732.
- Sakamoto, M., Suzuki, M., Umeda, M., Ishikawa, I., and Benno, Y. (2002) Reclassification of *Bacteroides forsythus* (Tanner et al. 1986) as *Tannerella forsythensis* corrig., gen. nov., comb. nov. *Int. J. Syst. Evol. Microbiol.*, **52**, 841–849.
- Suzuki, M., Nakagawa, Y., Harayama, S., and Yamamoto, S. (1999) Phylogenetic analysis of genus *Marinilabilia* and related bacteria based on the amino acid sequences of GyrB and emended description of *Marinilabilia salmonicolor* with *Marinilabilia agarovorans* as its subjective synonym. *Int. J. Syst. Bacteriol.*, **49**, 1551–1557.
- Suzuki, M., Nakagawa, Y., Harayama, S., and Yamamoto, S. (2001) Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: Proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amyolyticum* sp. nov. *Int. J. Syst. Evol. Microbiol.*, **51**, 1639–1652.
- Tamaki, H., Hanada, S., Kamagata, Y., Nakamura, K., Nomura, N., Nakano, K., and Matsumura, M. (2003) *Flavobacterium limicola* sp. nov., a psychrophilic, organic-polymer-degrading bacterium isolated from freshwater sediments. *Int. J. Syst. Evol. Microbiol.*, **53**, 853–857.
- Weller, R., Glockner, F. O., and Amann, R. (2000) 16S rRNA-targeted oligonucleotide probes for the in situ detection of members of the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Syst. Appl. Microbiol.*, **23**, 107–114.
- Zhu, F., Wang, S., and Zhou, P. (2003) *Flavobacterium xinjiangense* sp. nov. and *Flavobacterium omnivorum* sp. nov., novel psychrophiles from the China No.1 glacier. *Int. J. Syst. Evol. Microbiol.*, **53**, 853–857.