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Intestinal, segmented, filamentous bacteria in a wide range of vertebrate species

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Summary

Segmented, filamentous bacteria (SFBs) form a group of bacteria with similar morphology and are identified on the basis of their morphology only. The relationships of these organisms are unclear as the application of formal taxonomic criteria is impossible currently due to the lack of an *in vitro* technique to culture SFBs. The intestine of laboratory animals such as mice, rats, chickens, dogs, cats and pigs is known to harbour SFBs. To see whether this extends to other animal species, intestines from 18 vertebrate species, including man, were examined. SFBs were detected with light microscopy in the cat, dog, rhesus monkey, crab-eating macaque, domestic fowl, South African claw-footed toad, carp, man, laboratory mouse and rat, wood mouse, jackdaw and magpie. These results suggest that non-pathogenic SFBs are ubiquitous in the animal kingdom. Among apparently identical animals, there was considerable variation in the degree of SFB colonization. It is suggested that SFB colonization could serve as a criterion of standardization of laboratory animals.

Keywords: Segmented filamentous bacteria (SFBs); Vertebrates; Intestine; Microflora; Standardization

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Segmented, filamentous bacteria (SFBs) are non-pathogenic, autochthonous microbes and have been found in the ileum of mice and rats from various laboratories throughout the world (Davis & Savage, 1974; Chase & Erlandsen, 1976; Ferguson & Birch-Andersen, 1979; Garland *et al.*, 1982; Martin & Holland, 1984; Tannock *et al.*, 1984; Koopman *et al.*, 1987). SFBs have never been cultured *in vitro*, and thus never classified and characterized biochemically. They may or may not be related to each other. They are identified with the use of electron or light microscopy on the basis of their large dimension (up to a length of more than 1 mm), unique morphology (filaments, segmentation, spore-formation) and specific habitat (attachment to the ileal mucosa). A morphological and ecological similarity exists between mouse and rat SFBs, but it is likely that they represent at least 2 different bacterial species. Mouse-derived SFBs do not colonize the rat ileum and vice versa (Tannock *et al.*, 1984; Koopman *et al.*, 1984). Mainly on the basis of chance observations, it would seem that SFB-like bacteria occur in the intestine of vertebrates such as frog and toad (tadpole), domestic duck, domestic fowl (Fuller & Turvey, 1971), guineapig, zebra, dog, cat, sheep, pig (Sanford, 1991) and vervet monkey (*Cercopithecus aethiops*) and in the intestine of invertebrates such as myriapod, termite, cockroach, beetle and isopod (Klaasen *et al.*, 1992). Thus, although no systematic investigations were carried out, it appears that SFB-like bacteria are ubiquitous. In the gut of termites, Margulis *et al.*

(1990) have observed a group of filamentous, spore-forming bacterial species belonging to the genus *Arthromitus*. *Arthromitus*-like bacteria may be taxonomically related to the SFB-like bacteria in various vertebrates and invertebrates.

From the point of view of laboratory animal science, SFBs are of considerable interest. They have been suggested to contribute to the colonization resistance of the small intestine to pathogenic bacteria (Merrell *et al.*, 1979; Roach & Tannock, 1979; Garland *et al.*, 1982). The appearance of SFBs in the ileum of mice can vary greatly between apparently identical individuals and mean incidence of SFB colonization in apparently identical groups can vary considerably (Klaasen *et al.*, 1990, 1991b). The variability of SFB colonization may well affect other parameters. Theoretically, standardization of animals and their environment should reduce the variability of results, but standardization is limited to the sources of variation that we know and can control (Beynen, 1991). Causes of variation in SFB colonization in mice are diet, strain and housing (Klaasen *et al.*, 1990, 1991c; Koopman *et al.*, 1989). There may be more.

Table 1. Characteristics of animals examined for the presence of SFBs

Species	*Breed or strain*	*Number of individuals studied (♀ + ♂)*	*Age*	*Origin*[a]	*Type of housing*[b]	*Type feed*[c]
Guineapig (*Cavia porcellus*)	Dunkin Hartley	4, 3	2–8 months	A	cc	a
Golden hamster (*Mesocricetus auratus*)		15, 12	1½–5 months	A	cc	b
Rabbit (*Oryctolagus cuniculus*)	New Zealand White	7, 5	1½–9 months	A	cc	c
Cat (*Felis catus*)	European shorthair	0, 27	6–9 months	A	c	d
Dog (*Canis familiaris*)	Beagle	1, 4	3½–11 years	A	c	e
		5, 18	3–6 months	B	c	e
Goat (*Capra hircus*)	Saanen	6, 0	1½–2 years	C	c	f
Horse (*Equus caballus*)	Shetland pony	1, 1	20 years	A	c	g
Rhesus monkey (*Macaca mulatta*)		5, 0	2 weeks–15½ years	A	cc	h
Crab-eating macaque (*Macaca fascicularis/irus*)		3, 2	2 years	A	cc	h
		10, 13	15 months–15 years	D	cc	h
Domestic fowl (*Gallus domesticus*)	White Leghorn	30, 0	8–9 weeks	E	SPF	j
		24, 0	10–15 weeks	F	cc	j
South-African claw-footed toad (*Xenopus laevis*)		1, 3	5–7 years	A	c	k
Carp (*Cyprinus carpio*)		10[d]	1 year	G	c	k

[a]Explanation of symbols: A, Central Animal Laboratory, Catholic University of Nijmegen, The Netherlands (the cats had been bred under SPF conditions and were derived from ancestors obtained in 1975 from OLAC Western Ltd, Llandeilo, Pyfed, Wales, UK); B, housed at the Central Animal Laboratory, Nijmegen, but obtained at the age of 2½ months from Intervet International BV, Boxmeer, The Netherlands; C, various goat breeding farms in The Netherlands; D, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands; E, Intervet International BV, Boxmeer, The Netherlands; F, Poultry Health Institute, Doorn, The Netherlands (chickens derived from various commercial breeders in The Netherlands); G, Biological Institute, Catholic University of Nijmegen, The Netherlands.
[b]Explanation of symbols: cc, clean-conventional; c, conventional; SPF, specified pathogen-free.
[c]Explanation of symbols: a, guineapig diet LC23-B; b, rat–mouse–hamster diet RMH-TM; c, rabbit diet LK-04; d, cat diet LF-32; e, dog food D.B.; f, goat pellet; g, grass and hay; h, primate diet G.O.; j, various commercial diets; k, trout food, diets a–e and h were obtained from Hope Farms BV, Woerden, The Netherlands, diet f from Sluis, Veghel, The Netherlands, and diet k from Trouw Diervoeders BV, Putten, The Netherlands.
[d]Sex unknown.

Table 2. Characteristics of mice and rats screened for the presence of SFBs

Species	*Strain*	*Supplier*[a]	*Age*	*Immunological state*	*Type of housing*[b]	*Type feed*[c]
Mouse (*Mus musculus*)	BALB/c ABom nu/nu	A	1–7 months	Athymic	bm	a
Mouse	Crl:CD-1 (ICR)BR nu/nu	B	2½–5½ months	Athymic	bm	a
Mouse	Crl:NIH3BR ('scid')	B	5 months	Few LAK cells	bm	a
Rat (*Rattus norvegicus*)	LEW:Han	C	4½–5 months	Euthymic	cc	b
Rat	CDF(F-344) CRlBR	B	5 months	Euthymic	cc	b
Rat	F-344 rnu/rnu	D	5 months	Athymic	bm	a
Rat	Cpb:WU	E				
	'freezing rats'		3–9 months	Euthymic	cc	b
	'fleeing rats'		*idem*	*idem*	*idem*	*idem*

[a]Explanation of symbols: A, Bomholtgaard Breeding and Research Center Ltd, Ry, Denmark; B, Charles River Wiga GmbH, Sulzfeld, Germany; C, Zentralinstitut für Versuchstierzucht, Hannover, Germany; D, Harlan OLAC Ltd, Bicester, UK; E, Home-bred Cpb:WU rats derived from ancestors obtained in 1980 from the central laboratory animal facility of TNO, Zeist, The Netherlands. The production and characteristics of the 'freezing' and 'fleeing' rats have been described by Cools *et al.* (1990).
[b]Explanation of symbols: bm, barrier-maintained; cc, clean-conventional.
[c]Explanation of symbols: a, SRM-GS; b, RMH-TM. The diets were purchased from Hope Farms BV, Woerden, The Netherlands.

The objective of the present study was systematically to examine, on the basis of morphological characteristics, the occurrence of SFBs in a wide range of vertebrate species used as laboratory animals. In addition to species that have been studied previously (guineapig, cat, dog, domestic fowl, rat, mouse), we have looked at others not hitherto studied, namely the hamster, rabbit, goat, horse, rhesus monkey, crab-eating macaque, South-African claw-footed toad and carp. In addition, we searched microscopically for the presence of SFBs in human intestinal samples and those from one wild mammal and two wild birds, which were offered for postmortem examination. In athymic mice, the population density of SFBs may be lower than in euthymic mice (Davis & Balish, 1979). We therefore investigated selected asymptomatic mice and rats with a genetically impaired immune system.

Materials and methods

Origin of samples

Intestinal samples from individuals of 14 laboratory animal species were examined. Tables 1 and 2 present characteristics of the animals studied. The mice and rats were selected on the basis of their specific characteristics. There were two strains of athymic (nude) mouse and one strain of 'scid' mouse with a reduced number of lymphokine-activated killer (LAK) cells (Kamel-Reid & Dick, 1988). We used a strain of rat (Lew:Han; Lewis) with an increased susceptibility to autoimmune diseases (Holda & Swanborg, 1980) and a Fischer strain (CDF(F-344)CrlBR), which is frequently used as a control for Lewis rats (Davis *et al.*, 1985). The F-344 rnu/rnu strain is a strain of Fischer nude rats. There were two types of Cpb:WU (Wistar) rats with a genetically determined difference in behavioural responses, so-called 'freezing' and 'fleeing' rats (Cools *et al.*, 1990). The animals studied and shown in Tables 1 and 2, were derived from control groups of current experiments.

Human intestinal samples were derived from 6 patients either examined or operated at the University Hospital of Nijmegen. In one patient (no. 1), an ileal biopsy was taken. In the other 5 patients parts of the gut were surgically removed. None of the patients had been treated with antimicrobial drugs shortly before collection of samples. However, two patients (nos. 5 and 6) were treated with corticosteroids prior to resection. Patients' characteristics were as

follows: patient no., sex, age in years, intestinal disease, parts of the intestine examined for SFBs:

1 male, 67, intestinal polyps, ileum;
2 female, 55, intestinal polyps, ileum plus colon ascendens, transversum and descendens;
3 female, 46, colitis ulcerosa, ileum plus colon ascendens, transversum and descendens;
4 female, 57, colitis ulcerosa, ileum plus colon ascendens and transversum;
5 male, 33, colitis ulcerosa, colon transversum and sigmoid colon;
6 male, 20, Crohn's disease, jejunum plus ileum and caecum

Three wild animals, found in a diseased state or killed by accident, were examined. These were a wood mouse (*Apodemus sylvaticus*) and a jackdaw (*Corvus monedula*) both discovered in the region of Nijmegen, and a young magpie (*Pica pica*) from the area near Hamburg, Germany.

Preparation of mucosal smears

The animals in Tables 1 and 2 were euthanized as follows. Inhalation of carbon dioxide was used for guineapigs, golden hamsters, mice and rats. Rabbits, cats, dogs, goats, ponies, rhesus monkeys and crab-eating macaques were killed by intravenous administration of an overdose of barbiturate. Domestic fowl were killed by cervical dislocation, and toads and carp by decerebration. The distal half of the small bowel and the complete caecum (if present) were removed. From 3 dogs and 2 goats, palatine tonsils were also removed, and from the South African claw-footed toads, carp, jackdaw and magpie, the colon and rectum were collected.

Each tonsil was removed from the oral cavity and the mucosal surface was rubbed vigorously on a microscope slide over a surface of 3 cm^2. Each tonsil was cut into three equal parts and all cut surfaces were similarly rubbed on a slide. The intestinal segments to be examined were opened lengthwise. The contents were gently removed with a pair of forceps. Pieces of gut wall, each with a surface of circa 1 cm^2, were removed at a rate of 1 piece per 1–10 cm of gut, depending on the length of the gut. The method

Table 3. Incidence of SFB-positive animals from 12 vertebrate species

		SFB-positive animals Number/total number of animals				
		Small bowel				
Species[a]	*Origin*[b]	♀	♂	*Caecum*	*Other sites*	*%*
Guineapig	A	0/4	0/3	0/7		0
Golden hamster	A	0/15	0/12	0/27		0
Rabbit	A	0/7	0/5	0/12		0
Cat	A		1/27	0/27		4
Dog	A	1/1	1/4	0/4	2/3[c]	40
	B	3/5	4/18	0/23		30
Goat	C	0/6		0/6	0/2[c]	0
Pony	A	0/1	0/1	0/2		0
Rhesus monkey	A	1/5		1/5		20
Crab-eating macaque	A	0/3	0/2	0/5		0
	D	3/10	3/13	2/23		26
Domestic fowl	E	22/30		30/30		100
	F	13/24		22/24		92
South African claw-footed toad	A	0/1	1/3	n.c.[d]	1/4[e]	25
Carp	G	4/10[f]		n.c.	4/10[e]	40

[a]Characteristics of animals studied are described in Table 1.
[b]See Table 1.
[c]Tonsillar mucosa.
[d]n.c. = no caecum.
[e]Colonic mucosa.
[f]Sex unknown.

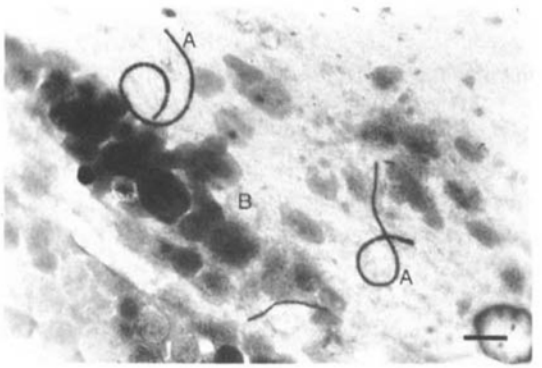

Fig. 1. Light microscopy of a mucosal smear from the ileum of a cat. (A), Segmented, filamentous bacterium (SFB). (B), gut material consisting of epithelial cells and mucus. Bar = 12 μm.

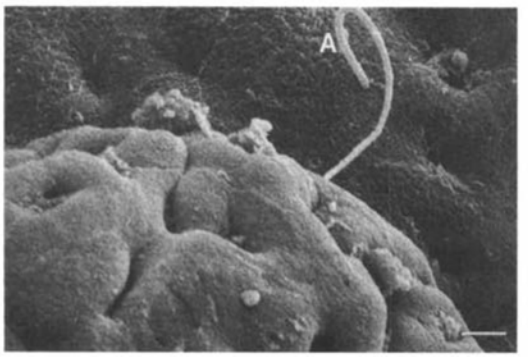

Fig. 2. Scanning electron microscopy of the tip of an ileal villus of a cat. (A), SFB attached to the villous epithelium. Bar = 18 μm.

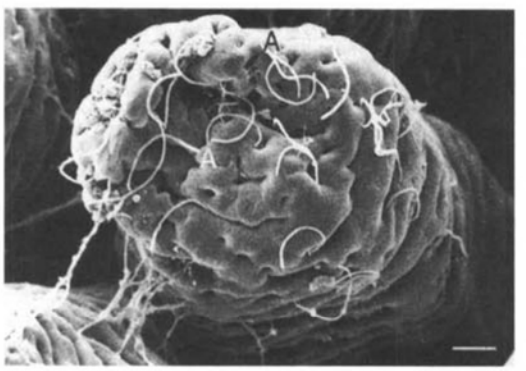

Fig. 3. Scanning electron microscopy of an ileal villus of a dog. (A), Attachment sites of SFBs. Bar = 16 μm.

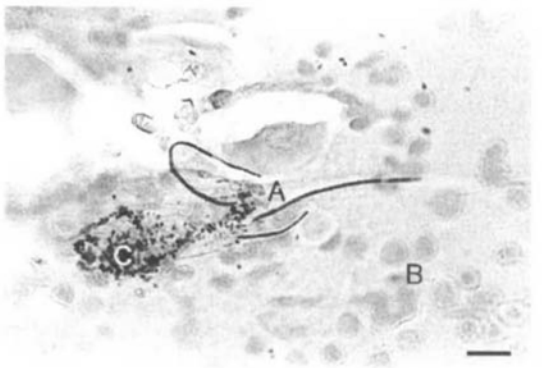

Fig. 4. Light microscopy of a smear from the tonsillar mucosa of a dog. (A), SFB. (B), Tonsillar material consisting of epithelial cells and mucus. (C), Accumulation of cell debris and bacteria. Bar = 12 μm.

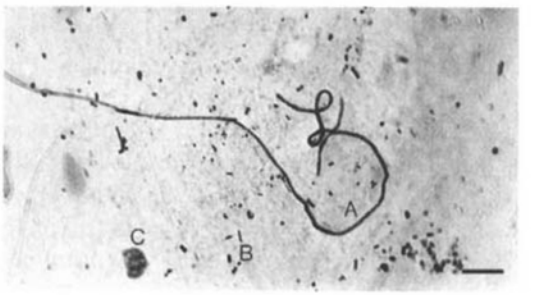

Fig. 5. Light microscopy of a mucosal smear from the ileum of a crab-eating monkey. (A), SFB. (B), Rod-shaped and coccoid, intestinal bacteria. (C), Mucosal material. Bar = 12 μm.

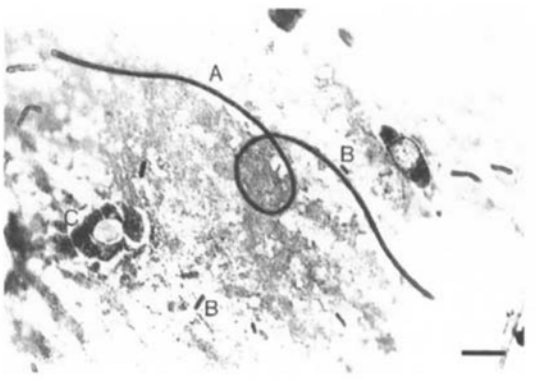

Fig. 6. Light microscopy of a mucosal smear from the small intestine of a South-African claw-footed toad. (A), SFB. (B), Rod-shaped, intestinal bacteria. (C), Damaged epithelial cell. Bar = 12 μm.

used for the preparation of small intestinal samples from mice and rats has been described previously (Koopman *et al.*, 1986). The mucosal surface of each piece of gut wall was rubbed on a slide as described above. Tonsillar and intestinal mucosal smears were heat fixed and Gram-stained.

Light microscopy

SFBs were identified on the basis of their morphology only. Thus, the presence of filaments with a diameter of 0·8–1·6 μm, length of 2–1000 μm and a segmented appearance were taken as evidence of SFBs. Bacteria with SFB-like morphology were identified by light microscopic examination of the mucosal smears at a magnification of 1000×. In each smear, 100 fields from 5 randomly chosen areas of the slide (20 fields per area) were examined. The incidence of SFB-positive animals was determined. For individual mice and rats, the mean number of SFB-positive fields per smear (SFB score, ranging from 0–100) was calculated. For mice with SFB scores ranging from 20 to 60, the within-smear variation of positive fields/20 fields is on average 30% (coefficient of variation). Light micrographs of SFBs in mucosal smears were taken with a Leitz Orthoplan® photo microscope.

Scanning electron microscopy

From selected SFB-positive animals, samples of gut wall were taken to examine the mucosa and adhering SFBs with the use of scanning electron microscopy. These samples were obtained as described above, flushed with a 0·9% (w/v) NaCl solution, stretched on filter paper and fixed for 24 h at 20 °C in a solution of 2% (w/v) glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·4, 320 mOsmol). After a brief rinse with 0·1 M cacodylate buffer (pH 7·4, 20 °C), the specimens were post-fixed for 3–5 h at 20 °C in Palade fixative (2% OsO_4 in 0·6 M veronal acetate buffer, pH 7·4). Thereafter, they were dehydrated in a graded series of ethanols or acetones and critical-point dried. After coating with a 30 nm gold layer in a Polaron E5100® sputter coater, the specimens were studied in a

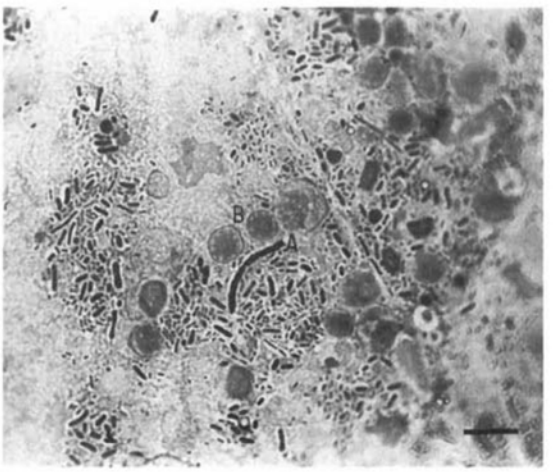

Fig. 7. Light microscopy of a mucosal smear from the colon of a carp. (A), SFB. (B), Mucosal material consisting of epithelial cells and mucus. Bar = 12 μm.

Table 4. SFB colonization of the small intestine in various mouse and rat strains

Species[a]	*Strain*[a]	*SFB-positive animals* Number/total number of animals	%	*SFB score*[b] $\bar{X}$	Range
Mouse	BALB/c ABom nu/nu	19/19	100	26	2–67
Mouse	Crl:CD-1 (ICR)BR nu/nu	5/5	100	26	8–60
Mouse	Crl:NIH3BR xid/xid ('scid')	4/4	100	32	6–39
Rat	LEW:Han	3/8	38	1	0–3
Rat	CDF(F-344)CrlBR	3/5	60	2	0–4
Rat	F-344 rnu/rnu	3/5	60	4	0–9
Rat	Cpb:WU				
	'freezing' rats	10/14	71	6	0–20
	'fleeing' rats	10/14	71	4	0–22

[a]Characteristics are given in Table 2.
[b]Number of SFB-positive fields per 100 fields as examined in each of five mucosal smears (magnification, 1000×).

Philips SEM 500® scanning electron microscope. Micrographs were taken at 12–15 kV and at magnifications varying between 160× and 3000×.

Results

For 12 vertebrate species, the incidence of SFB-colonization based on light microscopic findings is given in Table 3. SFB-positive animals were found in the following species tested: cat, dog, rhesus monkey, crab-eating macaque, domestic fowl, South-African claw-footed toad and carp. Figures 1–7 show light or scanning electron micrographs of selected, SFB-positive animals. In all SFB-positive animals, except for 17 out of the 54 chickens, SFBs were detected in the small intestine. All SFB-positive chickens had SFBs in the caecum (Table 3). In 2 of 9 positive dogs, SFBs were not only detected in the small intestine, but also in a smear from the tonsillar mucosa. The positive toad showed SFBs both in the small intestine and colon (Table 3). The location of SFBs in carp was either the small intestine or colon.

SFBs were found in both sexes of dog and crab-eating macaque. In the group of four toads one of three males was positive but the female was negative (Table 3). The numbers of animals examined were too small to demonstrate sex differences in SFB colonization. The SFB scores of positive animals in Table 3 varied between one and 79. Within groups of positive animals, there was no uniform SFB localization pattern in the small intestine (data not shown).

Light microscopic examination of the distal small intestine of mice and rats from various strains showed a higher incidence of SFBs in mice than in rats (Table 4). For each mouse strain tested, the incidence was 100%. For the rat strains tested, the incidences ranged between 38 and 71%. Mean SFB scores were higher for the mice than rats.

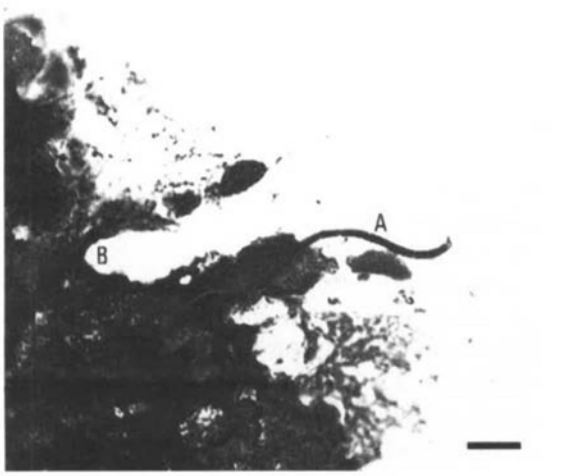

Fig. 8. Light microscopy of a mucosal smear from the ileum of a human adult. (A), SFB attached to the epithelium. (B), Mucosal material consisting of epithelial cells and mucus. Bar = 12 μm.

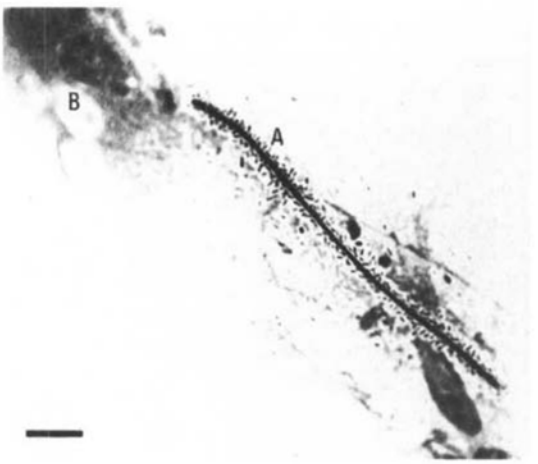

Fig. 9. Light microscopy of a mucosal smear from the ileum of a wood mouse. (A), SFB surrounded by numerous rod-shaped bacteria. (B), Mucosal material consisting of epithelial cells and mucus. Bar = 12 μm.

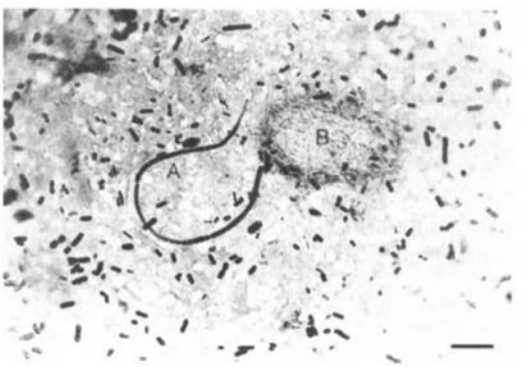

Fig. 10. Light microscopy of a mucosal smear from the small intestine of a jackdaw. (A), SFB. (B), Mucosal material consisting of epithelial cells and mucus. Bar = 12 μm.

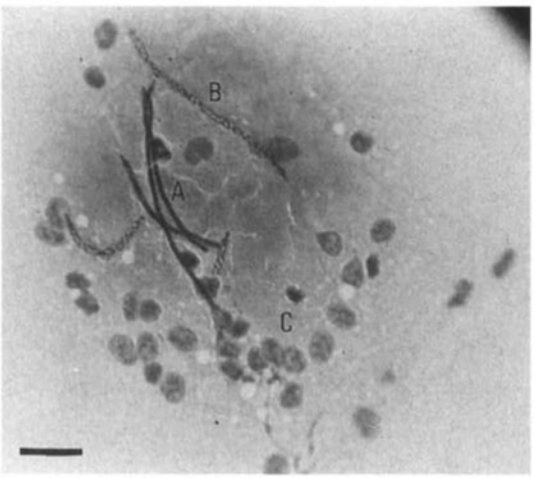

Fig. 11. Light microscopy of a mucosal smear from the small intestine of a magpie. (A), SFB, slender and dark-coloured. (B), SFB, broader and light-coloured. (C), Mucosal material consisting of epithelial cells and mucus. Bar = 12 μm.

One of the 6 human patients tested had SFBs in the ileum as shown by light microscopy (Fig. 8). From the positive patient, an ileal biopsy without abnormalities had been obtained. The three wild animals examined (wood mouse, jackdaw and magpie) had SFBs in the small intestine, as demonstrated by light microscopy (Figs. 9–11).

Discussion

Examination of intestinal samples from animals of 13 out of the 18 species tested, revealed the presence of SFBs. There was a great variation in the degree of SFB colonization between and within species. SFBs have been shown to attach preferentially to ileal Peyer's patches or caecal tonsils, suggesting a functional relationship between SFBs and mucosa-associated lymphoid tissue (Abrams, 1977; Glick *et al.*, 1978; Owen & Nemanic, 1978; Garland *et al.*, 1982; Tannock *et al.*, 1984). However, in asymptomatic mice and rats with an impaired immune system, relatively large numbers of SFBs were found. This could imply that attachment of SFBs to the Peyer's patch epithelium is not dependent on the presence of T cells under the epithelium. Colonization density of SFBs was higher in the mice than in the rats examined. The idea of the possible relationship between SFBs and lymphoid tissue is supported by the observations that in dogs SFBs were associated with tonsillar mucosa, and in chickens with caecal tonsils.

The present study supports the chance observations that SFB-like bacteria can occur in a wide variety of vertebrate host species (Klaasen *et al.*, 1992). As far as we know, this study documents for the first time that SFBs were sought in small numbers of hamster, rabbit, goat and pony but were not found, whereas they were found in man, rhesus monkey, crab-eating macaque, South-African claw-footed toad, carp, wood mouse, jackdaw and magpie. The light microscopic observation of SFBs in man, rhesus monkey and crab-eating macaque is in accord with the scanning electron microscopic observation of SFBs in another primate, the vervet monkey (Bruorton, 1991). However, our observations have to be interpreted carefully because the microbes found in the latter 8 species have not been examined by scanning or transmission electron microscopy. It should also be emphasized that the inability to detect mucosa-associated SFBs could relate to the gut being only poorly or irregularly colonized by SFBs (Davis & Balish, 1979). When sampling of SFB-positive animals takes place more than 3 h postmortem SFBs may not be detected (Davis, 1980; own unpublished observations), possibly due to destruction of SFB attachment sites by autolytic enzymes. Except for the SFB-positive sample, the other human ileal samples were derived from diseased intestines. Thus, the low incidence of SFBs in the group of 6 patients may not be representative of colonization in man.

Colonization of SFBs is dependent on a number of host-related and environmental factors. Different strains of mice and rats can differ markedly in the density of SFB populations (Klaasen *et al.*, 1990). Age has been shown to be an important determinant of SFB colonization in mice (Davis & Savage, 1974; Klaasen *et al.*, 1990), rats (Garland *et al.*, 1982) and chickens (Glick *et al.*, 1978). In mice, diets with different composition affect SFB population densities (Koopman *et al.*, 1986; Klaasen *et al.*, 1991b,c).

Stress factors that tend to lower numbers of SFBs in mice are overcrowding, lack of bedding, continuous light and housing at high temperature (Koopman *et al.*, 1989). Antimicrobial treatment of mice may reduce SFB colonization or even eliminate SFBs from the intestine (Klaasen *et al.*, 1991e). Thus, we described the characteristics of the animals examined as carefully as possible (Tables 1 and 2). However, even in apparently identical individual animals or groups of animals, SFB colonization can differ considerably (Klaasen *et al.*, 1991b) due to factors unknown. The variation of SFB colonization in apparently identical animals may serve as a measure of standardization of animals for experimentation. Reproducibility from one experiment to another of SFB colonization could imply reproducibility of other parameters as well.

Thus, mucosa-associated, SFB-like bacteria occur in various host species, including man and at least 2 other primate species. Margulis *et al.* (1990) have identified 22 microscopically distinguishable, symbiotic arthromitids in 9 different arthropod hosts. Possibly, in the course of evolution, symbiotic intestinal SFBs evolved together with their hosts into a stable mutualistic relationship. Biochemical studies and bacterial DNA/RNA analysis will be necessary to unravel the taxonomy of SFBs. SFBs do not disappear, nor do they cause clinical disease when their host lacks certain immunological functions. We may, therefore, conclude that SFBs are non-pathogenic, ubiquitous, autochthonous gut inhabitants. This raises the question of their significance to the host. With the use of germ-free and SFB-mono-associated mice (Klaasen *et al.*, 1991d), the influence of SFBs on gastrointestinal colonization resistance and mucosal immunity can be investigated. If SFBs appear to be beneficial to the host, then investigations on human SFBs may become relevant to human health.

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