Taxonomic Study of *Bacteroides clostridiiformis* subsp. *clostridiiformis* (Burri and Ankersmit) Holdeman and Moore and of Related Organisms: Proposal of *Clostridium clostridiiformis* (Burri and Ankersmit) comb. nov. and *Clostridium symbiosum* (Stevens) comb. nov.

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Ninety-three strains of *Bacteroides clostridiiformis* subsp. *clostridiiformis* (Burri and Ankersmit) Holdeman and Moore, *B. biacutus* (Weinberg and Prévot) Holdeman and Moore, *Fusobacterium symbiosum* (Stevens) Moore and Holdeman, and related organisms, of which 75 were freshly isolated from the fecal material of humans, were studied systematically. They were differentiated into two genera, *Clostridium* and *Bacteroides*. We propose that, of the spore-forming strains, those having a guanine plus cytosine (G+C) content of about 49% to be considered *C. clostridiiformis* (Burri and Ankersmit) comb. nov., for which ATCC 25537 is designated as the neotype strain; those having a G+C content of about 56% be considered a new species in the genus *Clostridium*; and those having a G+C content of about 46% and producing butyric acid as a major product be considered *C. symbiosum* (Stevens) comb. nov., for which ATCC 14940 is designated as the neotype strain. Of the nonsporeforming strains, we propose that those having a G+C content of about 53% be considered a new species in the genus *Bacteroides*.

The systematic positions of the anaerobic, gram-negative, motile or nonmotile, pointed-ended organisms resembling *Bacteroides clostridiiformis* subsp. *clostridiiformis* are difficult to determine, particularly since a number of phenotypically similar strains of this sort have recently been shown to have spores (1, 15, 16, 32, 34).

In the course of a study on the intestinal flora of humans and animals, a large number of strains that appear to be identical, or closely related, to the above-mentioned organisms were isolated. However, most of the isolates were found to have spores, indicating that they be placed in the genus *Clostridium*. A study was undertaken, therefore, to determine the taxonomic positions of the organisms and of fresh isolates from human feces.

MATERIALS AND METHODS

Bacterial strains. The 93 strains studied and their sources are listed in Table 1. Of these, 75 strains were freshly isolated, in amounts of 10^5 to 10^8 per g, from feces of clinically normal adult humans. Three strains from three separate sources,

ATCC 25537, VPI 0316-1, and CIP T90, all originated from one strain isolated from a young calf designated T90 by Bryant et al. (7).

Isolation and maintenance of cultures. Fecal materials emulsified in an anaerobic buffer solution were plated on modified Eggerth-Gagnon (EG) agar, as described by Mitsuoka et al. (21), and on the EG agar or the basal medium of Schaedler et al. (33), with 0.001% (wt/vol) cephalothin (Eli Lilly and Co.) and 0.0005% (wt/vol) crystal violet (Merck) added as selective agents. Incubation was carried out at 37 C for 3 days in an anaerobic, steel-wool jar (25) filled with an atmosphere of 100% CO₂. Organisms with pointed ends were selected. Strains were maintained on prereduced EG liver agar slants with H₂CO₃-CO₂ buffer (21) and stored at 4 C. New transfers were usually made at 3-month intervals.

Biochemical tests. For preparation of inocula, peptone-yeast extract (PY) broth (15) with 1% (wt/vol) glucose and 4% (vol/vol) Fildes peptic digest of horse blood (Fildes solution) (12) was used (designated PYFG broth). The medium was autoclaved at 115 C for 20 min and, after boiling, was aseptically supplemented with 0.05 ml of ascorbic acid-cysteine solution per 3 ml immediately before use. The solution was prepared by adding 35.2 g of ascorbic acid, 10.6 g of Na₂CO₃, and 2.0 g of cysteine HCl·H₂O to 100 ml of distilled water; it was then autoclaved and stored at 4 C under a 100% CO₂ gas phase. Incubation was carried out in the anaerobic, steel-wool jar. The inocula were prepared by making one to two preliminary transfers in PYFG broth.

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TABLE 1. *Strains studied*^a

Our grouping		Strain and laboratory no.	Source and date	Obtained from:
Group	Sub-group			
Sporeformer (<i>Clostridium</i>)	Ia	<i>Fusiformis biacutus</i> CIP 2071 ^b	Appendix, 1926	A. R. Prévot
		<i>Fusiformis polymorphus</i> CIP 141D ^b		M. Sebald
		<i>Fusiformis polymorphus</i> CIP 143D ^b	Appendix, 1928	M. Sebald
		<i>Ristella clostridiiformis</i> CIP 3219 ^b		A. R. Prévot
	Ib	ECC24; ECC30; ECC34; ECC35; ECC47; ECC1-8; ECC1-9; ECC-DY2; ECC-DY6; ECC-KK5; ECC-YB4	Feces of adult man, 1972-1973	Our collection
		<i>Bacteroides clostridiiformis</i> subsp. <i>clostridiiformis</i> ATCC 25537 ^b	Rumen, young calf, 1958	ATCC
		<i>Bacteroides clostridiiformis</i> subsp. <i>clostridiiformis</i> VPI 0316-1 ^b	Rumen, young calf, 1958	W. E. C. Moore
		<i>Clostridium microsporum</i> 2013173 ^b	Pus from appendix	H. Beerens
		<i>Eggerthella clostridiiformis</i> 354 ^b		Ella M. Barnes ^c
		<i>Fusiformis biacutus</i> CIP T90 ^b	Rumen, young calf, 1958	M. Sebald
		<i>Fusiformis biacutus</i> CIP 4309	Abdominal abscess, 1967	M. Sebald
		<i>Ristella biacutus</i> CIP 4476	Scrotal abscess, 1967	M. Sebald
		ECC3; ECC16; ECC25; ECC36	Feces of adult man, 1972-1973	Our collection
	Ic	<i>Bacteroides biacutus</i> ATCC 25551 ^b	Feces of a Japanese- Hawaiian male, 1972	ATCC
		<i>Bacteroides clostridiiformis</i> subsp. <i>gir-</i> <i>ans</i> VPI C16-3-1 ^b		W. E. C. Moore
		<i>Bacteroides necrophorus</i> NCTC 7155 ^b	Perirenal abscess Feces of adult man, 1972-1973	Ella M. Barnes
		ECC5; ECC6 ^b ; ECC9; ECC11; ECC12; ECC13; ECC14; ECC15; ECC18; ECC22; ECC23; ECC26; ECC27; ECC28; ECC29; ECC31; ECC32; ECC33; ECC37; ECC38; ECC39; ECC40; ECC41; ECC42; ECC43; ECC44; ECC45; ECC46; ECC48; ECC49; ECC1-1; ECC1-2; ECC1-4; ECC1-5; ECC2-1 ^b ; ECC2-2; ECC2-3; ECC3-1; ECC3-2; ECC3-3; ECC3-4; ECC4-1; ECC4-3; ECC4-4; ECC5-1; ECC5-2; ECC5-3; ECC5-4; ECC6-1; ECC6-2; ECC6-3; ECC6-4; ECC-DY1; ECC-CK1; ECC-CK2; ECC-KH1; ECC-KK1; ECC-KK2; ECC-YB2; ECC-YB3		Our collection
	II	<i>Bacteroides biacutus</i> VPI 4969-1 ^b	Rumen, young calf, 1958	W. E. C. Moore
	III	<i>Fusiformis biacutus</i> 25 ^b	Symbiote with <i>En-</i> <i>tamoeba histoly-</i> <i>tica</i> , 1948	Ella M. Barnes
		<i>Bacteroides symbiosus</i> ATCC 14940 ^b		ATCC
Nonsporeformer (<i>Bacteroides</i>)		EBF 77/78 ^b	Cecum of chicken, 1970	Ella M. Barnes

^a ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England; CIP, Collection de l'Institut Pasteur, Paris, France; VPI, Virginia Polytechnic Institute, Blacksburg, Va.

^b Strains for which G+C contents were determined in the present study.

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The basal medium for most biochemical tests was PY broth with 4% (vol/vol) Fildes solution added (designated PYF broth). For the biochemical tests, 0.1 ml of inoculum per 3 ml of test medium was usually used. All of the liquid media were held in a

boiling-water bath for 20 min before use. Incubation was at 37 C in the anaerobic, steel-wool jar with an atmosphere of 90% N₂-10% CO₂ for 10 days except where otherwise stated.

Acid production from carbohydrates was deter-

mined in PYF broth containing 0.5% (wt/vol) of the carbohydrates to be tested. Among them, arabinose, xylose, ribose, rhamnose, sorbose, mannose, fructose, and maltose were sterilized by filtration and added aseptically to the basal medium. The pH value was measured directly in culture tubes by a long combination electrode (automatic pH measuring system, model CAT-160, Toa Electronics Ltd., Tokyo). The final pH value was determined in PYFG broth after incubation for 10 days.

The formation of gas in PYF and PYFG broths was detected by the appearance of bubbles in Durham tubes. Indole production in PYF broth and/or indole nitrate medium (BBL) with 0.05% (wt/vol) cysteine HCl·H₂O added was determined with Kovac reagent. Litmus milk (Difco) with 0.05% (wt/vol) cysteine HCl·H₂O added was used to test acid and curd production and digestion. Growth stimulation or inhibition by bile was determined by adding 2% (wt/vol) Oxgall (Difco) to PYFG broth. Lipase activity was detected in PYFG agar plates with 1% (vol/vol) glycerol tri-*n*-butyrate added as described by Hugo and Beveridge (17). A positive reaction was detected by a zone of clearing around the colony. The glutamic acid decarboxylase test was performed by using Bio-Test Disc (Eiken Co. Ltd., Tokyo).

Production of H₂S was tested by the methods of (i) Holdeman and Moore (15), (ii) Barnes and Impey (2), (iii) Werner (39), and (iv) Dowell and Hawkins (10). With the strains tested, the decreasing order of the occurrence of positive reactions using the four methods was as follows: i ≥ ii ≥ iii > iv, and that of the strength of the reaction was iii ≥ i ≥ ii > iv. Considering these results, H₂S production was determined in this study mainly by method i.

The media and methods described by Holdeman and Moore (15) were used for the following tests: nitrate reduction, gelatin liquefaction, urease activity, casein digestion, lecithinase and catalase reactions, ammonia and acetylmethylcarbinol productions, starch and esculin hydrolyses, and threonine deamination.

Detection of motility and flagella staining. Motility was observed under a phase-contrast microscope after 4 to 10 h of incubation at 30°C in the fluid of syneresis of medium 10 (9) or prereduced EG liver agar slants and was also detected by diffusion in sloppy PYF agar (0.25% [wt/vol] agar) after 7 days of incubation at 30°C. For flagella staining, organisms were grown in PYF broth, with ascorbic acid-cysteine solution added, at 30°C for 24 h. After 0.5 ml of the neutralized formalin was added to 3 ml of the broth cultures, the cells were centrifuged, washed twice with distilled water, and stained by the method of Nishizawa and Sugawara (18).

Spore formation test. Organisms were grown in chopped-meat (CM) agar slants (15) at 30°C and stained periodically up to 3 weeks with the Gram or spore stains. They were also incubated on EG agar plates at 37°C for 2 days, allowed to stand at room temperature for about 2 to 3 weeks, and then subjected to staining. Prereduced starch broth (15) was usually inoculated with growth from CM agar slants incubated for 10 days at 30°C and heated at 50, 60, 70, and 80°C for 10 min in a water bath. In some

cases, growth from 5- and 20-day-old CM slants and/or 3-week-old EG plates was used.

Fermentation products. Strains were grown for 3 days in PYFG broth. Alcohols, volatile acids, and nonvolatile acids were analyzed by gas chromatograph (no. GC-4BMPF, Shimadzu Ltd., Tokyo, equipped with a hydrogen flame ionization detector) methods. Media samples (2 ml) were acidified with 50% aqueous H₂SO₄, and the products were extracted three times with ethyl ether. The extract was appropriately concentrated by a gentle stream of N₂ and analyzed for alcohols and volatile fatty acids on a column packed with FAL-M (F-L) (Wako Pure Chemical Ltd., Tokyo). Nonvolatile acids were analyzed on the same column; the same operating conditions were used after methylation by diazomethane. Formic acid was not detected by this method.

DNA base composition. Purified deoxyribonucleic acid (DNA) was prepared by the method of Marmur (19) modified to include phenol extraction steps in place of the chloroform-isoamyl alcohol deproteinization treatment. The moles percent guanine plus cytosine (G+C) content of the DNA was determined by the thermal melting point method of Marmur and Doty (20) with an automatic recording spectrophotometer (Komatsu Electronics, Tokyo).

RESULTS

The 93 strains studied were divided into sporeformers (*Clostridium*) and nonsporeformers (*Bacteroides*). The sporeformers were further divided into three distinct groups of species, mainly on the basis of G+C content and glucose fermentation products. Their characteristics are listed in Table 2.

Characters common to all strains. All of the strains studied had certain features in common. They were obligately anaerobic, gram-negative rods with pointed ends; they occurred singly, in pairs, and sometimes in short or long chains. Filamentous forms were often seen, except for strains of *Clostridium* group III. Cells grown on BL agar were larger or stouter than those grown on EG agar. Round, central to terminal spores were formed in CM agar slants and EG agar plates, except for strain EBF 77/78; they were inconsistent in other media tested. They resisted heating at 70°C for 10 min but not consistently at 80°C for 10 min (usually after incubation for 10 days in CM agar slants). Surface colonies on EG agar incubated for 3 days were grayish to whitish, convex, circular, translucent, smooth (sometimes with a slightly irregular surface and margin), and had a whitish-gray or reddish mottled center when viewed through a dissecting microscope. They were 1.0 to 2.5 mm in diameter, except the strains of group III, which formed smaller colonies (0.5 to 1.0 mm).

***Clostridium* group I.** The strains of *Clostrid-*

TABLE 2. Characteristics of 18 strains of *B. clostridiiformis*, *B. biacutus*, *F. symbiosum*, and related organisms, and of 75 human fecal isolates^a

Characteristics	Sporeformer							Nonspore-former
	Group I (<i>C. clostridiiformis</i>)				Group II (<i>Clostridium</i> sp.)	Group III (<i>C. symbiosum</i>)		(<i>Bacteroides</i> sp.)
	Subgroup a	Subgroup b	Subgroup c	Neotype strain ATCC 25537	VPI 4969-1	All strains	Neotype strain ATCC 14940	EBF 77/78
No. of strains	15	11	63	1	1	2	1	1
Spore formation	+	+	+	+	+	+	+	—
Spore location	ST	SCT	ST	SCT	ST	S	S	—
Heat resistance at: 70 C for 10 min	+	+	+	+	+	+	+	—
80 C for 10 min	v	v	v	+	—	v	+	—
Final pH in PYFG	5.3–5.8	5.3–5.8	5.3–5.8	5.3–5.6	5.3–5.8	5.3–6.0	5.3–6.0	5.3–5.5
Motility:								
0.25% agar	—+	—+	v	—	—	+	+	—
Microscopy	+-	+-	+-	—	—	+	+	—
Flagellum	+-	+-	+-	—	—	+	+	—
G+C content (mol%)	48.5 ^b	49.0 ^c	48.9 ^c	48.8	56.3	46.2 ^d	46.1	53.2
Products from PYFG:								
Alcohols	—	(+)-	-(+)	(+)	Trace	(+)	(+)	—
Acetic acid	++	++	++	++	++	++	++	++
Propionic acid	—	—	—	—	—	—	—	—
Butyric acid	—	—	—	—	—	++	++	—
Lactic acid	++	(+)+	++(+)	(+)	++	(+)	(+)	(+)
Succinic acid	—	—	-(+)	—	—	—	—	—
Threonine conversion	—+	—	—+	—	+	+	+	—
Lactate utilization to propionic acid	—	—	—	—	—	—	—	—
Glutamic acid decar- boxylase	—	—	—	—	—	—	—	—
Nitrate reduction	—	—	—w	—	—	—	—	—
Production of:								
Indole	+	—	—	—	+	—	—	—
H ₂ S	+	+w	+w	+	w	w	w	—
Acetylmethylcarbinol	+	+	+w	+	+	—	—	+
NH ₃	—	—	—	—	—	+	+	—
Hemolysis	—	—	—	—	—	—/b ^e	—	b ^e
Neutral red reduction	+	+	+	+	+	+	+	+
Gas from:								
PYFG	—+	+	+	+	+	+	+	+
PYF	—w	—w	w—	w	+	w/—	w	w
Litmus milk:								
Acid	—+	+—	+—	+	+	—/+	—	+
Curd	—	+—	+—	w	+	—/+	—	—
Digestion	—	—	—w	—	—	—	—	—
Gelatin liquefaction	—	—	—	—	—	—	—	—
Growth in 20% bile	+s	s	s—	s	w	—	—	—
Acid from:								
Arabinose	+	+	+	+	+	+	+	—
Xylose	+	+	+	+	—	+	+	—
Rhamnose	—+	+	+—	+	—	—	—	—
Sorbose	—	—w	+—	—	—	—	—	—
Ribose	+—	+	+w	+	+	—	—	w
Glucose	+	+	+	+	+	+	+	+
Mannose	+w	+	+	+	+	+	+	—
Fructose	+	+	+	+	+	+	+	—
Galactose	+	+	+	+	+	+	+	+

TABLE 2—Continued

Characteristics	Sporeformer							Nonspore-former
	Group I (<i>C. clostridiiformis</i>)				Group II (<i>Clostridium</i> sp.)	Group III (<i>C. sym- biosum</i>)		(<i>Bacteroides</i> sp.)
	Subgroup a	Subgroup b	Subgroup c	Neotype strain ATCC 25537	VPI 4969-1	All strains	Neotype strain ATCC 14940	EBF 77/78
Sucrose	+	+	+	+	+	—	—	+
Maltose	+	+	+	+	+	—	—	+
Cellobiose	—w	+—	—+	+	—	—	—	—
Lactose	—w	+—	+—	+	+	—/+	—	+
Trehalose	+	+—	+	—	+	—	—	+
Melibiose	+—	+—	+—	+	—	—	—	—
Raffinose	+—	+—	+—	+	—	—	—	+
Melezitose	—	—	+	—	—	—	—	—
Dextrin	—	—+	—+	—	—	—	—	—
Starch	—	—	—	—	—	—	—	—
Glycogen	—	—+	—+	—	—	—	—	—
Inulin	—	—	—+	—	—	—	—	—
Glycerol	A1	A1	A1 ^f	A1	— ^g	—	—	—
Mannitol	—	—	—	—	— ^g	w	w	—
Sorbitol	—	—	—+	—	—	—	—	—
Inositol	—	—	—	—	—	—	—	—
Esculin	—	—	—	—	—	—	—	—
Salicin	—+	—+	—w	—	—	—	—	—
Amygdalin	—	—w	—	—	—	—	—	—
Starch hydrolysis	—w	—+	—w	+	—	—	—	—
Esculin hydrolysis	+—	+	+—	+	—	—	—	—

^a All strains were negative for catalase, urease, lecithinase, and lipase (Tributylin) reactions and casein digestion. Symbols: +, reaction positive for >90% of strains; —, reaction negative for >90% of strains; w, reaction weak for >90% of strains; v, reaction variable within the strains or in a strain; b, beta hemolysis; s (bile cultures), stimulated; A1 (glycerol cultures), pH higher than that of control, usually 0.3 to 0.7 pH unit; +—, —w, etc., first reaction most frequent, 10 to 40% of strains had second reaction; —/+, w/—, etc., different reactions for two strains. Spore locations: C, central; S, subterminal; T, terminal. First location is most frequent; last is least frequent. These differences in location were usually observed also in a strain. Products: ++, major product; (+), moderate product. Formic acid was not detected by the method used.

^b Average value of four strains.

^c Average value of five strains.

^d Average value of two strains.

^e Occasional slight beta hemolysis.

^f Except for strain *B. biacutus* ATCC 25551, which showed a negative reaction.

^g With occasional weak reaction.

ium group I had a variety of biochemical reactions from strain to strain. Three subdivisions (Ia, Ib, and Ic) were recognized within this group by the production of indole and fermentation of melezitose.

Cells on EG agar (3 days) were 0.4 to 0.7 by 1.0 to 3.0 μ m. The motility varied from strain to strain in the subgroups. All of the named strains were nonmotile, except for strains CIP 141D, CIP 143D, and VPI C16-3-1. The motility of the two CIP strains, each with a subpolar flagellum, was only sluggish and was not detected in sloppy-agar medium. The VPI strains were peritrichous (two to six flagella) and were more

motile; however, no motility was detected in the sloppy agar. In contrast, most of the fresh isolates, which were also peritrichous (two to eight flagella), were actively motile.

Of group I, subgroup Ib strains formed spores the most easily. *C. microsporum* 2013173 persistently showed spores in all media used and had relatively short cells. The spore formation of two strains of subgroup Ic, ATCC 25551 and VPI C16-3-1, was not demonstrated easily. The EG agar cultures often had to be left at room temperature for more than 30 days to detect spores, and even then only a small number of spores were seen.

Slight to moderate growth occurred in PYF broth. PYFG cultures had moderate to abundant growth, usually with a smooth sediment. One of the distinctive features common to all strains of this group (except for strain ATCC 25551 [subgroup Ic]) was that the pH of their glycerol cultures was usually 0.3 to 0.7 pH unit higher than that of control cultures. Only two strains of subgroup Ic, ATCC 25551 and VPI C16-3-1, produced succinic acid in moderate amounts.

G+C contents of the DNAs of 14 strains were in the range of 48 to 50 (average about 49) mol% by T_m .

Clostridium group II. *Clostridium* group II is comprised of strain VPI 4969-1. Cells on EG agar (3 days) were 0.5 to 0.8 by 2.0 to 5.0 μm . Moderate growth occurred in PYF broth. PYFG broth cultures had abundant growth with a smooth sediment.

Clostridium group III. *Clostridium* group III consisted of two strains, 25 and ATCC 14940. Cells on EG agar (3 days) were 0.5 to 0.6 by 1.5 to 2.0 μm . They were actively motile and peritrichous. The number of flagella varied from cell to cell. Most cells of strain 25 had one to three flagella; most cells of strain ATCC 14940 had no flagella, but some had six to eight. Diffused colonies were often observed. Slight growth occurred in PYF broth. PYFG cultures had moderate growth, with a smooth sediment. They were less active in carbohydrate fermentation than strains of the other two groups.

Bacteroides group. The *Bacteroides* group was composed of only strain EBF 77/78. Its characters were nearly as described by Barnes and Impey (3). It was nonmotile, nonsporulating, and rod shaped with pointed ends. Cells on EG agar (3 days) were 0.5 to 0.8 by 2.0 to 10.0 μm . Some cells grown in media with fermentable carbohydrates had an intracellular granule- or vacuole-like appearance. After incubation for 10 days in CM agar slants, they resisted heating at 50 C for 10 min but not at >60 C for 10 min.

Another colonial type sometimes appeared on EG agar. It was raised, opaque, and nearly white. This type of colony contained comparatively small cells with no other distinct characteristics. There was slight growth in PYF broth and moderate growth in PYFG broth. H_2S production was negative in the four different media, whereas it was positive in the original description.

DISCUSSION

The taxonomy of *B. clostridiiformis* subsp. *clostridiiformis* (14), *B. biacutus* (14), *Fusobacterium symbiosum* (23), and related organisms

is extremely complicated. The chronological relationships of their taxonomy are presented in Fig. 1.

A nonmotile form was first described as *Bacterium clostridiiforme* by Burri and Ankersmit (8) in 1906. Weinberg and Prévot (38) reported another nonmotile form as *Fusobacterium biacutum*, which was subsequently called *Fusiformis biacutus* by Hauduroy et al. (13). Prévot (26, 28, 29) listed these two nonmotile organisms as *Ristella clostridiiformis* and *F. biacutus*, respectively. Beerens et al. (5) showed that the *F. biacutus* strains could be divided into two distinct groups: type I, producing acetic and formic acids and not converting threonine to propionic acid; and type II, producing acetic, formic, and butyric acids and converting threonine to propionic acid. In 1962, Beerens et al. (6; H. Beerens, M. M. Castel, and P. Fiévez, International Congress for Microbiology, Montreal, 1962, p. 120) reclassified the type I strains, together with the strains of *R. clostridiiformis*, as *Eggerthella clostridiiformis* but retained the type II strains in *F. biacutus*. In contrast, Sebald (34) reclassified the strains of *F. biacutus* (Beerens types I and II) and *R. clostridiiformis* as *Ristella biacuta* since they were nonmotile and had G+C contents of more than 40%. Two strains having a G+C content of 30% were classified as *Sphaerophorus clostridiiformis*.

On the other hand, in 1948 Prévot (28) described a new motile form with pointed ends as *Fusocillus pedipedis* (Beveridge). He also studied the strains of streptobacilli isolated by Shaffer et al. (35), which were subsequently designated *B. symbiosus* by Stevens, and classified three of them (LSU, Reeves, and ATCC) into *F. pedipedis*. Sebald (34) studied nine strains, including these three strains, all descended from *B. symbiosus*, and classified two motile strains (Reeves and ATCC) as *Zuberella pedipedis*, three nonmotile strains (LSU, S5, and S6) as *R. biacuta*, and four motile strains (S1, S2, S3, and S4) as *Z. plauti* (Seguin). Moore and Holdeman (24), however, reclassified at least two of them (LSU and Reeves) as *Fusobacterium symbiosum* (Stevens) on the basis of the ability to produce butyric acid and on their opinion that the morphological characteristics were so dissimilar to those depicted by Beveridge for his motile fusiform that it was highly improbable that they were representatives of the Beveridge organism.

Recently, Holdeman and Moore (16, 22) proposed, based on the overall classification of these kinds of organisms, that, of the strains producing acetic and formic acids, strains having a G+C content of 53 to 55% be considered *B. clostridiiformis* subsp. *clostridiiformis*, strains

Fig. 1. Chronological relationships of the classification of *B. clostridiiformis* subsp. *clostridiiformis*, *B. biacutus*, *F. symbiosum*, and related organisms. The transfers of the strains of *B. symbiosum* and closely related organisms are indicated along the lines.

having a G+C content of about 40% be considered *B. biacutus*, and strains producing butyric acid as a major product be considered fusobacteria.

All of these organisms have been described as nonsporeformers. However, several authors (1, 16, 32) have detected spores in numerous strains of the organisms. We also detected spores in all of the strains tested except EBF 77/78. They therefore belong to the genus *Clostridium*. However, they differ from other clostridia primarily in morphology. Furthermore, as compared with five clostridial strains having high G+C ratios (*C. innocuum* ATCC 14501 [44.5%], *C. indolis* ATCC 25771 [44.7%], *C. oroticum* ATCC 13619 [43.7%], *C. sphenoides* ATCC 19403 [41.0%], and *C. nexile* ATCC 27757 [40.5%]), they could also be clearly distinguished in several biochemical characteristics, fermentation products, and/or G+C contents.

Three divisions (groups I, II, and III) were recognized within the sporeformers on the basis of G+C contents, fermentation products, and other biochemical properties. Most strains so far classified as *B. clostridiiformis* subsp. *clostridiiformis* and *B. biacutus* could be included in group I. The previous descriptions of the two species (5, 8, 14, 15, 26, 27, 30, 34, 38) also indicate that they are similar to each other except for a few differences like production of indole and milk curd and fermentation of lactose and melezitose. Differences were also observed among strains of group I. However, the biochemical properties also varied, so that such differences could hardly be regarded as differential characteristics of the species level. We believe that the strains (group I) should be placed in a species. Furthermore, Reinhold et al. (32) have suggested that some of the strains are identical to *C. microsporum* Spray (36). *C. microsporum* 2013173 also belonged to group I. Its characteristics were as described originally by Spray (36, 37), except that it fermented lactose, rhamnose, and raffinose, formed milk curd, and had no motility. In spite of such deviation, the strain may be regarded as a representative of *C. microsporum* since carbohydrate fermentation and motility tend to vary among the strains of group I. According to the *Bacteriological Code* (11), the name *Clostridium clostridiiformis* (Burri and Ankersmit) comb. nov. may be proposed for group I strains since the specific epithet *clostridiiformis* is an earlier epithet than *biacutus* and *microsporum*. Since the type strain is not available, ATCC 25537 (= VPI 0316-1 = CIP T90) is proposed as the neotype strain.

Strain VPI 4969-1, placed in group II, was

clearly separated from the group I strains by the G+C content (56.3%) and xylose and glycerol fermentation. Sebald (34) determined the G+C content of this strain to be 55.2%, close to our value. Hence, this strain may represent a new species in the genus *Clostridium*. We think that it might be classified as *C. biacutum* (Weinberg and Prévot) because its characteristics are, for the most part, in agreement with the original description of *F. biacutum*, although more strains must be studied before the establishment of such a new combination.

Two strains, *B. symbiosus* ATCC 14940 and *F. biacutus* 25 (Beerens type II), could be placed in group III. Sebald (34) and Barnes and Goldberg (1) also reported that the two strains were similar in their ability to produce butyric acid and in other properties. We found their G+C contents to be about 46%. This value is considered compatible with those given by Sebald (34) (47.8 and 44.6% for the two strains, respectively, and 46% for the *Z. pedipedis* strains [Reeves and ATCC]). Reeves et al. (31) reported a similar value (48%) for *B. symbiosus*. Moreover, when the descriptions of *B. symbiosus*, *F. biacutus* (Beerens type II), *Z. pedipedis*, and the two strains reported here are compared with each other, no differences are recognized except for a few characters like milk coagulation and motility. Sebald (34) considered the two strains (LSU and 25) to be nonmotile and placed them in *R. biacuta*; however, in the present study they were found to be actively motile. Moore and Holdeman (22, 23) reported that *F. symbiosum* was variable in motility. Consequently, motility is judged not to be a reliable characteristic for differentiation of this kind of organism. Furthermore, Reinhold et al. (32) detected spores in cultures of strain 25. We detected spores in two strains (25 and ATCC 14940). Thus, it is highly likely that a majority of strains of *Fusobacterium symbiosum*, *Z. pedipedis*, and *F. biacutus* (Beerens type II) will form a new and well-defined species in the genus *Clostridium*. In addition, the characteristics of the Beerens type II strains are so different from the original and later descriptions of *F. biacutus* that they can not be regarded as representatives of the species. Therefore, the name *Clostridium symbiosum* (Stevens) comb. nov. is proposed for this group of organisms in view of the above-mentioned opinion of Moore and Holdeman (24) concerning the relationship between the Beveridge fusiform and *F. symbiosum*. The type strain is not available. We propose that ATCC 14940 be designated as the neotype strain.

Among the strains tested, EBF 77/78 was the

only strain that did not form spores. This strain was called *Bacteroides clostridiiformis* subsp. *clostridiiformis* by Barnes and Impey (3, 4). However, in spite of the morphological similarity, it differed from the strains of *Clostridium* groups I, II, and III by the following characteristics: G+C content of 53.2%; inability to form spores; no growth in the presence of brilliant green (0.001%); and no acid production from arabinose, fructose, and mannose. Furthermore, this strain is considered to be distinguished from *Bacterium clostridioforme* (8) (*Bacteroides clostridiiformis* subsp. *clostridiiformis*) for the following reasons. (i) The former was described as growing poorly in medium 10 without supplement and was liable to die rapidly in stock culture, whereas the latter was described as growing well in dextrose media. (ii) Spores of the latter were probably overlooked by the original authors, as has been the case with other strains of this sort, particularly in media containing relatively large amounts of fermentable carbohydrate. (iii) Sources are different; i.e., the former is from the avian cecum and the latter is from the cattle. Therefore, EBF 77/78 may form a new species in the genus *Bacteroides*. In addition, Barnes and Impey (4) recently reported another nonsporeforming, gram-negative, rod with pointed ends isolated from an avian cecum. The authors considered them to be different from the strains used in this study. Hence, none of nonsporeforming strains that conform to the descriptions of *B. clostridiiformis* subsp. *clostridiiformis*, *B. biacutus*, and *F. symbiosum* has been found among the presently described organisms. This fact also supports the appropriateness of the transfer of the three above-mentioned organisms to the genus *Clostridium*.

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REPRINT REQUESTS

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