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Isolation of *Acidaminococcus fermentans* and *Megasphaera elsdenii* from Normal Human Feces

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Fecal bacterial cultures from 40 normal humans yielded *Megasphaera elsdenii* from four individuals and *Acidaminococcus fermentans* from 10 individuals, with two individuals having both organisms.

Two gram-negative anaerobic cocci, *Acidaminococcus fermentans*, described by Rogosa (7), and *Megasphaera elsdenii*, recently renamed and redescribed by Rogosa (8), have been isolated from animals. Our laboratory noted an isolate of *A. fermentans* from a sample of normal human feces in 1972 (2). We also noted that *A. fermentans* had been isolated from 6 of 22 normal humans and *M. elsdenii* from 2 of 22 humans (P. T. Sugihara, V. L. Sutter, H. R. Attebery, K. S. Bricknell, and S. M. Finegold, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 116, 1973). Werner (11) indicated *M. elsdenii* as a probable inhabitant of the intestines of 4 of 16 normal humans.

Fecal samples from 40 normal humans were processed either in an anaerobic chamber (1) or with bench anaerobic methods (anaerobic jars with 10% H_2, 10% CO_2, and 80% N_2). Samples were homogenized and serial dilutions were made in WAL-1 diluent (9) in the chamber, and 0.05% yeast extract diluent was made on the bench. Samples of dilutions (0.1 ml) were streaked onto various media by the rotator-pipette method (H. R. Attebery and W. T. Carter, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 11, 1972). Kanamycin-vancomycin blood agar (KV), rifampin-vancomycin blood agar (RV), *Veillonella* agar with neomycin (VN), *Bifidobacterium* selective agar, egg yolk agar with neomycin (EYA-N), and blood agar were made as indicated in the Wadsworth Anaerobic Bacteriology Manual (9). Eugonagar with maltose and blood agar with neomycin (NEO) were made as described by Finegold (4). Other media used were: *Fusobacterium* agar (FM) (6) and China-blue agar (10).

Incubation was carried out at 37 C for 2 to 3 days. Counts of organisms were corrected to the dry weight of the specimens.

Six isolates of *M. elsdenii*-like organisms, from four individuals, and 15 isolates of *A. fermentans*-like organisms, from 10 individuals, were recovered. Both types were found in two individuals. Table 1 shows the counts of these isolates on various media. Six of 10 media used contained antibiotics (NEO, VN, EYA-N, FM, KV, and RV). Without the use of a battery of media, a number of the isolates of both organisms would have been overlooked. Colony counts (Table 1) indicate that these organisms are usually present in relatively large numbers.

The organisms were characterized by tests and criteria as outlined in the Wadsworth Anaerobic Bacteriology Manual (9) and the V.P.I. Anaerobic Laboratory Manual (5). Their characteristics were compared with those of type strains ATCC 20585 (*A. fermentans*), ATCC 25940 (*M. elsdenii*), ATCC 17745 (*Veillonella alcalescens*), and ATCC 10790 (*V. parvula*).

Some distinctive characteristics of these organisms are shown in Table 2. Susceptibility to colistin and resistance to vancomycin are important features in distinguishing the *M. elsdenii* which commonly stains gram-positive, although it is really gram-negative, on the basis of cell wall composition (8). The *Veillonella* species also gave similar results. Although all reported strains of *A. fermentans* have been indole negative, it is interesting to note that, in this study, three isolates from one individual were identical to *A. fermentans*, except that they were all indole positive. These are probably variants of *A. fermentans*. Analysis of volatile fatty acids produced as major end products in peptone-yeast extract broth and peptone-yeast extract-glucose broth was done by gas chromatography (3).

The finding of *A. fermentans* in 25% and *M.*

TABLE 1. *Recovery media and colony counts of A. fermentans-like and M. elsdenii-like organisms*[a]

Medium	*A. fermentans*	*M. elsdenii*
Kanamycin-vancomycin blood agar	10^{9}[b], 10^{8}, 10^{6}, 10^{7}	—[c]
Neomycin blood agar	10^{8}, 10^{9}, 10^{7}	10^{8}
Veillonella-neomycin	10^{8}, 10^{7}	10^{8}
Fusobacterium agar	10^{8}, 10^{6}	—
Eugonagar-maltose	—	10^{9}, 10^{8}
Blood agar	10^{9}, 10^{8}	—
Bifidobacterium selective	—	10^{8}
China-blue agar	10^{7}	—
Egg-yolk-neomycin	—	10^{5}
Rifampin-vancomycin blood agar	10^{3}	—

[a] Counts are in grams per dry weight of feces to the nearest log base 10.

[b] Each figure is the count from one specimen; for example, *A. fermentans* was isolated on kanamycin-vancomycin agar from four individuals at the counts noted.

[c] Indicates organism was not recovered.

TABLE 2. *Certain distinctive characteristics of gram-negative anaerobic cocci*[a]

Organism	Disk tests		Carbohydrate fermentation	Nitrate reduction	Volatile fatty acid end products	
	Vancomycin (5 μg)	Colistin (10 μg)			PY	PYG
A. fermentans	R	S	0	0	AB	AB
M. elsdenii	R	S	+	0	0	BC
Veillonella alcalescens and *V. parvula*	R	S	0	+	AP	AP

[a] PY, Peptone-yeast extract broth; PYG, peptone-yeast extract-glucose broth; R, resistant; S, sensitive; A, acetic acid; B, butyric acid; C, caproic acid; and P, propionic acid. Carbohydrates were glucose, maltose, fructose, and sucrose.

elsdenii in 10% of normal human fecal samples indicates that these organisms are part of the normal intestinal flora.

We have recently isolated *A. fermentans* from a closed abdominal abscess and *M. elsdenii* from a putrid lung abscess (by transtracheal aspiration) as part of mixed anaerobic and facultative flora in both instances. This suggests that these organisms may play a role in human pathological processes as well.

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LITERATURE CITED

1. Aranki, A., S. A. Syed, E. B. Kenny, and R. Freter. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. **17**:568-576.
2. Attebery, H. R., V. L. Sutter, and S. M. Finegold. 1972. Effect of a partially chemically defined diet on normal human fecal flora. Amer. J. Clin. Nutr. **25**:1391-1398.
3. Bricknell, K. S., and S. M. Finegold. 1973. A simple, rapid method to process and assay fatty acids and alcohols by gas chromatography. Anal. Biochem. **51**:23-31.
4. Finegold, S. M. 1970. Isolation of anaerobic bacteria, p. 265-279. *In* J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
5. Holdeman, L. V., and W. E. C. Moore (ed.). 1972. V.P.I. anaerobic laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg.
6. Ninomiya, K., S. K. Ohtani, H. Kamiya, K. Ueno, S. Suzuki, and T. Inoue. 1972. Simple and expedient methods of differentiation among *Bacteroides*, *Sphaerophorus* and *Fusobacterium*. Jap. J. Med. Sci. Biol. **25**:63-73.
7. Rogosa, M. 1969. *Acidaminococcus* gen. n., *Acidaminococcus fermentans* sp. n., anaerobic gram-negative diplococci using amino acids as the sole energy source for growth. J. Bacteriol. **98**:756-766.
8. Rogosa, M. 1971. Transfer of *Peptostreptococcus elsdenii* Guitierrez et al. to a new genus, *Megasphaera* [*M. elsdenii* (Guitierrez et al.) comb. nov.] Int. J. Syst. Bacteriol. **21**:187-189.
9. Sutter, V. L., H. R. Attebery, J. E. Rosenblatt, K. S. Bricknell, and S. M. Finegold. 1972. Anaerobic bacteriology manual. University of California Extension Division, Los Angeles.
10. Van der Wiel-Kortanje, J. A. A., and K. C. Winkler. 1970. Medium for differential count of the anaerobic flora in human feces. Appl. Microbiol. **20**:168-169.
11. Werner, H. 1973. *Megasphaera elsdenii*—a normal inhabitant of the human intestines? Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1 Orig. A **223**: 343-347.