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Cellular Fatty Acid Composition, Soluble-Protein Profile, and Antimicrobial Resistance Pattern of *Eubacterium lentum*

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Phenotypic heterogeneity among isolates of *Eubacterium lentum* has been recognized for many years. To better delineate their taxonomic relatedness, 29 clinical isolates of *E. lentum* were examined for soluble-protein content, cellular fatty acid profile, and antimicrobial resistance pattern in order to ascertain whether differences in these characteristics could be correlated with differences in biochemical activities. Among 29 isolates we could identify 6 that were different from all the others. These strains were coccobacilli with translucent colonies; they were catalase and H_2S negative, not fluorescent under UV light, and susceptible to beta-lactam drugs; growth was not stimulated by arginine; and fatty acid analysis revealed the presence of straight-chain fatty acids. The remainder of the strains, including the type species, were pleomorphic bacilli with speckled colonies and were catalase and H_2S positive; all but two were fluorescent under UV light; they were resistant to beta-lactam antibiotics; growth was greatly stimulated by arginine; and they demonstrated saturated branched-chain fatty acids. Our data suggest that *E. lentum* can be further differentiated into different types.

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Eubacterium lentum is a gram-positive, non-spore-forming obligate anaerobe isolated from normal human feces and infections in humans (8) which comprises 5 to 10% of anaerobic isolates in hospitals (3). The microorganism is characterized by a few positive biochemical reactions: namely, it is asaccharolytic, it reduces nitrate, and its growth is greatly enhanced by arginine (13). Recently *E. lentum* has been demonstrated to show orange to red fluorescence under UV light; this feature may represent a rapid tool for its laboratory identification (9). However, investigators have noted a heterogeneity among isolates of *E. lentum*. MacDonald et al. (7) identified two groups, i.e., *E. lentum* and phenotypically similar organisms, on the basis of production of two steroid-metabolizing enzymes (bile acid 3α-hydroxysteroid dehydrogenase and bile acid 12α-hydroxysteroid dehydrogenase) with minimal overlapping of biochemical characteristics. In fact, the synthesis of the steroid enzymes was positively correlated with stimulation by arginine, catalase activity, and H_2S production. These latter biochemical characteristics were also correlated with the production of red fluorescence (9). Moreover, Verhulst et al. (14) demonstrated that although they had similar G+C contents ranging between 63.7 and 69.1 mol%, steroid-producing and non-steroid-producing strains of *E. lentum* were characterized by different patterns of long-chain fatty acids.

Gas chromatographic analysis of cellular fatty acids and examination of protein patterns by polyacrylamide gel electrophoresis have been used taxonomically to distinguish among strains within a species and among organisms within a genus or family (2).

The aim of the present study was to better delineate the taxonomic relatedness of strains of *E. lentum* by examining soluble-protein contents, cellular fatty acid profiles, and antimicrobial resistance patterns to ascertain whether differences in these characteristics could be correlated with the previously described differences in biochemical activities.

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MATERIALS AND METHODS

Bacterial strains and their characterization. Strains used were from the culture collection of the Veterans Administration Wadsworth Anaerobic Bacteriology Laboratory, Los Angeles, Calif. At the time of isolation all strains were identified as *E. lentum* or "most likely *E. lentum*" by the criteria of Holdeman et al. (6). Reference strains of *E. lentum* (ATCC 25559 and ATCC 34055) were also included in the study. All the isolates have previously been examined by Mosca et al. (9) for the production of red fluorescence; 21 of 29 clinical strains as well as the type strains were red fluorescent. Among the eight isolates that did not exhibit red fluorescence, six were different from all the other strains with regard to colony morphology, cellular morphology, catalase and H_2S production, and arginine stimulation. The colony morphology of these six strains was punctiform and translucent in contrast to that of the other strains, which had circular, grey, speckled colonies. On Gram staining, these isolates appeared as coccobacilli, while the others were pleomorphic bacilli (Table 1).

Gas-liquid chromatography of cellular fatty acids. Each organism was subcultured in 10 ml of PRAS-PYG broth (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.) supplemented with arginine and incubated overnight at 35°C. Each of the six strains producing only scant turbidity was inoculated in four tubes to obtain a satisfactory cell pellet. Lysis of cells through saponification, methylation of fatty acids, and extraction of the methyl esters into the organic phase were achieved as previously described (4, 5). Samples were processed on a Hewlett-Packard 5890A gas chromatograph with a Hewlett-Packard 7673A automatic sampler and integrator. The chromatography unit was coupled to a computer with Microbial Identification System (MIS) automated software (5). For identification, the Virginia Polytechnic Institute broth-based anaerobe library was employed.

Polyacrylamide gel electrophoresis of cellular proteins. *E. lentum* strains were inoculated in 100 ml of prereduced brucella broth supplemented with arginine and incubated overnight at 35°C. The cultures were centrifuged and washed in Tris buffer (100 mM Tris, 10 mM $MgSO_4$, pH 7.4). The bacterial pellets were resuspended in 1.5 ml of Tris buffer, and cells were broken in a French pressure cell. The suspensions were centrifuged at 13,000 × *g* for 1 min to remove whole cells. The supernatants were transferred to microcentrifuge tubes, Triton X-100 medium (20% Triton X-100, 0.1 M HEPES, 0.1 M $MgCl_2$, pH 7.4) was added, and the tubes were placed in ice for 15 min. The tubes were then centrifuged, washed in Tris buffer, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10% separating gels. Samples were then resuspended in sample buffer (0.5 M Tris, glycerol, 10% sodium dodecyl sulfate, 2% mercaptoethanol, 0.05% bromophenol blue), loaded onto gels, and electrophoresed at 200 V for about 4 h.

Antimicrobial susceptibility testing. Ampicillin, cefotetan, cefotaxime, ceftriaxone, clindamycin, piperacillin, cefoxitin, metronidazole, imipenem, and chlor-

TABLE 1. Biochemical characteristics of *E. lentum*[a]

Category	Morphology	Strain[b]	Fluorescence production	Catalase activity	Arginine stimulation	H_2S production	Source[c]
A	Speckled-colony pleomorphic bacilli	ATCC 25559	+	+	3+	+	Infected rectal tumor
		ATCC 34055	+	+	3+	+	Abdominal wound
		7879	+	+	3+	+	Appendix tissue
		7889	+	+	3+	+	Rx maxill. sinus asp.
		7977	+	+	3+	+	Palate abscess
		8058	+	+	3+	+	Appendix tissue
		8060	+	+	3+	+	JP fluid
		8079	+	+	3+	+	Peritoneal fluid
		8086	+	+	3+	+	Peritoneal fluid
		8800	+	+	3+	+	Peritoneal fluid
		8816	+	+	3+	+	Unknown
		8849	+	+	3+	+	Unknown
		8923	+	+	3+	+	Appendix tissue
		8940	+	+	3+	+	Appendix tissue
		8955	+	+	3+	+	Appendix tissue
		8980	+	+	3+	+	Intrabdominal abscess
		8990	+	+	3+	+	Appendix tissue
		8996	+	+	3+	+	Peritoneal fluid
		9019	+	+	3+	+	Appendix tissue
		9025	+	+	3+	+	Peritoneal fluid
		6192	+	+	3+	+	Blood
		6246	+	+	3+	+	Blood
		6587	+	+	3+	+	Pericardial abscess
		8132	−	+	3+	+	Peritoneal fluid
		8186	−	+	3+	+	Transverse colon abscess
B	Translucent-colony coccobacilli	7741	−	−	1+	−	Peritoneal fluid
		7742	−	−	1+	−	Abdominal drainage
		8088	−	−	1+	−	Appendix tissue
		8413	−	−	1+	−	Appendix tissue
		8753	−	−	1+	−	Appendix tissue
		8782	−	−	1+	−	Appy abs fluid

[a] All strains were asaccharolytic and reduced nitrate.
[b] All strains except ATCC 25559 and ATCC 34055 were from Wadsworth Anaerobic Laboratory.
[c] Rx maxill. sinus asp., prescribed maxillary sinus aspirate; JP fluid, Jackson Pratt abdominal drainage; appy abs fluid, appendiceal abscess fluid.

amphenicol were selected either as representative of a class of compounds or as drugs for which MICs for quality control strains were published. MICs were determined by the National Committee for Clinical Laboratory Standards reference agar dilution method (10). Briefly, the antibiotic powders were reconstituted according to the manufacturer's instructions, and serial dilutions ranging from 256 to 0.015 μg/ml were prepared in brucella agar supplemented with vitamin K, hemin, and 5% laked sheep blood. The inoculum was prepared in an anaerobic chamber by suspending several colonies from a 72-h culture plate in brucella broth to achieve the visual turbidity of a 0.5 MacFarland standard. A Steers replicator (Craft Machine Co. Inc., Chester, Pa.) was used to apply the organisms to the plates for a final inoculum of 10^5 CFU per spot. Plates were incubated in an anaerobic atmosphere for 48 h. The MIC was defined as the lowest concentration of antimicrobial that resulted in no growth.

RESULTS

Cellular fatty acid analysis. The MIS is the first commercial system that takes into account the presence or absence of fatty acids, the nature of each acid, and their ratio for the classification of anaerobic organisms (1, 4, 12).

Long-chain components of 11 to 18 carbon atoms were identified in the bacterial extract of *E. lentum* with quantitative and qualitative differences among the strains (Table 2). Two types of patterns were observed. The first type (group A) was characterized by branched fatty acids, namely, 14:0 iso-fatty acid methyl ester (iso-FAME), 15:0 anteiso-FAME, 15:0 iso-dimethyl acetyl (iso-DMA), 15:0 anteiso-DMA, 16:0 iso-FAME, and 17:0 anteiso-DMA. On the basis of the above profile these strains were correctly identified as *E. lentum* by the MIS library. The branched-chain fatty acids were absent in the second type of pattern (group B), which was mainly characterized by 11:0 DMA, 12:0 FAME, 14:0 FAME, and 14:0 DMA. The six nonfluorescent strains of *E. lentum* presented this profile, which had no match or was wrongly identified as *Fusobacterium naviforme.*

Soluble-protein patterns. Cellular proteins were studied by electrophoretic separation. Each strain was characterized by a pattern containing about 30 discrete bands, most of which were relatively weakly stained. Although some qualitative and quantitative differences were observed, the grouping of the strains was difficult since considerable homogeneity in the protein profiles was present (data not shown).

Susceptibility testing. The strains of *E. lentum* showed two different antimicrobial susceptibility patterns (Table 3). In particular, group B strains were susceptible to ampicillin, cefotetan, cefotaxime, and ceftriaxone, in contrast to the strains belonging to group A. Although all the strains were susceptible to clindamycin, piperacillin, and imipenem, MICs for group B strains were lower than those for group A. No difference was observed when metronidazole and chloramphenicol were tested.

DISCUSSION

Early reports described gram-positive non-spore-forming bacilli similar to *E. lentum* but with some different phenotypic and genetic properties (7, 9, 14). In our study of 29 isolates of *E. lentum*, we could identify 6 (group B) that were different from all the others. These strains in fact were coccobacilli with translucent colonies, catalase and H_2S negative, not fluores-

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TABLE 2. Cellular long-chain components of 31 *E. lentum* strains

Strain	Proportion (% of total area of all peaks) of[a]:																				
	11:0 DMA	12:0 FAME	13:1 *cis*-12 FAME	13:0 iso-3OH FAME	14:0 iso-FAME	14:0 FAME	14:0 DMA	15:0 anteiso-FAME	15:0 iso-DMA	15:0 anteiso-DMA	15:0 iso-FAME	16:0 ALD	16:0 iso-FAME	16:0 FAME	UN-16.107 16i DMA	16:0 DMA	16:1 *cis*-9 FAME	17:0 anteiso-DMA	17:0 FAME	18:1 *cis*-9 FAME	18:0 FAME
ATCC 25559	—	—	2.18	—	12.9	4.46	4.28	30.25	1.71	3.43	—	3.79	2.83	4.44	11.34	11.74	1.32	4.6	—	312	2.75
ATCC 34055	—	—	2.9	2.1	17.8	6.6	6	31.2	1.6	4.7	1.4	3.6	1.4	4	4.6	7.2	1.2	1.2	—	1.8	—
7879	—	—	1.9	1.6	15.6	4.1	4	29.3	1.3	3.8	—	4.5	1.5	4.2	10	9.2	1	2.9	—	3.1	1.3
7889	—	—	1.2	1.3	11.2	4.1	3.7	23.2	1.2	3.8	—	2.9	1.7	7.1	10	11.7	1.1	3.3	—	7.55	2.9
7977	—	—	1.1	1.6	11.7	4.6	3.8	31.7	1.5	4.6	—	3.2	2.7	5	10.1	8.8	1.4	4	—	3.3	—
8058	—	—	1.2	1.9	14.9	3.5	3.2	29.8	1.2	4.8	—	2.49	3.5	4.7	9.7	7.7	1.4	2.8	—	3.6	2.9
8060	—	—	1.9	2.8	15.7	3.8	5.1	29.8	1.7	5.9	—	2.9	1	3	9.7	9.4	0.9	2.8	—	1.9	0.9
8079	—	—	2.4	2.9	18.3	4.7	5.5	34.1	2.1	7.1	1.4	3.5	1.8	2.6	4.7	5.8	—	1.5	—	—	—
8086	—	—	2.8	2.5	16.7	5.1	5.2	33.5	2.5	6.2	—	2.8	1.3	4.7	5.7	6.1	—	1.7	—	2.5	—
8800	—	—	2.6	2.6	16.2	5.3	6.1	34.4	2.9	5.4	1.9	4	0.8	1.9	5.1	5.9	—	1.9	—	—	—
8816	—	—	1.4	3.8	14.6	3.4	5.1	28.8	2.5	7.5	1.1	1.3	1.7	3.1	10.3	7.8	—	3.3	—	2.2	1.4
8849	—	—	1.6	2	12.9	4.7	5.6	23.9	1.5	5.1	—	2.8	1.6	5.5	9.8	12.1	—	2.6	—	4.8	2.7
8923	—	1.4	1.9	1.2	12.1	7.6	6	24.9	1	6	—	3.3	2.4	4.1	6.6	12.6	—	3	2	1.6	—
8940	—	—	—	3.9	18.7	5.2	6	29	2.1	6.5	—	2.6	0.8	2.3	4.9	4.5	0.7	1.3	1.1	1.2	0.6
8955	—	—	1.6	2	10.9	4.2	6.3	28.3	2.8	6.1	—	2.3	—	5.2	8.9	9.7	—	3.6	—	4.6	2.8
8980	—	—	2.1	3.5	15.1	4.1	4.6	29.1	2.3	7.3	—	2.4	1.4	3.7	9.2	10	—	2.8	—	1.6	2.7
8990	—	—	7.3	3.9	15.2	3.5	4.8	28.1	1.9	8	—	1.7	1.7	3.8	9	9.4	—	2.8	—	2	3
8996	—	—	2.4	—	17.5	4.7	4.6	31.8	3.3	7.7	2.6	2.7	1.9	3.9	4.6	6.4	—	—	—	2.5	—
9019	—	2.1	2.4	—	13.8	10.3	4.9	21.4	—	4.8	—	6.8	3	6.8	4.4	14.1	—	—	2.3	2.7	—
9025	—	—	2.4	2.9	22.2	3.6	4.6	25.2	—	4.2	—	4.4	7.7	4.9	9.3	6.6	1.6	1.6	—	4.1	—
6192	—	—	2.3	2.1	16.1	5.3	5.1	34.3	1.9	5.5	—	5.4	1.6	2.7	6	7.2	—	2.1	—	—	—
6246	—	—	1.2	2.4	16.9	3.8	4.1	34	1.1	5.3	—	3	3.1	3.9	8.2	8.7	—	2.3	—	1.3	—
6587	—	—	—	1.5	20	4.8	5	33.9	2	3.5	—	5.6	1.7	1.8	7.4	5.7	—	2.1	—	—	—
8132	—	—	1.4	1	11.9	3.8	3.1	38.1	1.6	3.4	—	5	3.6	4.7	9	10.1	—	3.3	—	2.6	—
8186	—	—	1.7	3.2	13.7	4.8	4.9	30.5	1.7	5	1.4	3.2	2.4	4.3	7.9	11.1	1.2	2.7	—	2	—
7741	2.7	5.3	10.3	—	—	23.6	22.3	—	—	—	—	5.5	—	6.8	—	18	13	—	1.6	4.1	3.5
7742	4.5	10	17.5	—	—	26	22	—	—	—	—	7.2	—	4.5	—	7.8	—	—	—	—	—
8088	3.3	10.4	15.7	—	—	28	17.8	—	—	—	—	7.3	—	3.4	—	9	0.8	—	4.5	1.5	—
8413	3.4	7.9	17.3	—	—	28.7	21	—	—	—	—	6	3.1	9.1	—	—	—	—	3	2.1	—
8753	3.2	8.9	12	—	—	25.7	17.3	—	—	—	—	6.8	—	5.2	—	15.7	—	—	2.5	3	—
8782	3.4	9	12.9	—	—	27	18.8	—	—	—	—	6	—	4.6	—	9.8	1.69	—	2.4	6.9	8.2

[a] Abbreviations: ALD, fatty aldehyde; iso and anteiso, branched-chain fatty acid; —, not found.

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TABLE 3. Antimicrobial susceptibility patterns of *E. lentum*[a]

Antimicrobial agent	MIC (μg/ml)	No. of strains	
		Group A	Group B
Ampicillin	2	25	0
	0.5	0	6
Cefotetan	128	4	0
	64	13	0
	32	8	0
	8	0	6
Cefotaxime	128	4	0
	64	3	0
	32	18	0
	0.5	0	6
Ceftriaxone	128	5	0
	64	14	0
	32	6	0
	1	0	6
Imipenem	2	6	0
	1	19	0
	0.06	0	6
Piperacillin	16	21	0
	8	4	0
	2	0	4
	1	0	2
Clindamycin	4	3	0
	1	9	0
	0.5	13	0
	<0.06	0	6
Metronidazole	2	12	5
	1	13	1
Chloramphenicol	16	20	3
	8	5	3

[a] All strains were β-lactamase negative.

cent, and susceptible to beta-lactam drugs, and their growth was not stimulated by arginine. The fatty acid profile revealed the presence of straight-chain fatty acids identical to those observed for steroid-inactive *E. lentum* strains (14). The remainder of the strains of *E. lentum* (group A), including the type species, were pleomorphic bacilli with speckled colonies, catalase and H_2S positive, and all (except for two strains) fluorescent, and their growth was greatly stimulated by arginine. Moreover, these strains were more resistant to beta-lactam drugs, and their cellular fatty acid pattern was characterized by saturated branched-chain fatty acids, with 15:0 as the most abundant.

Electrophoretic separation of cellular proteins represents a highly sensitive analysis providing distinctive phenotypic evidence of the similarity of strains (2). Although considerable homogeneity was observed in the protein profile, some qualitative and quantitative differences were detected in some species belonging to group B. Another method that has been used to analyze even minor genomic variation between strains is the restriction endonuclease analysis of bacterial chromosomal DNA. The pattern of DNA bands obtained with a given enzyme is a reproducible feature of a DNA and therefore has potential for typing closely related bacteria (11). We tested some endonucleases (*Hin*dIII, *Msp*I, and *Bsx*II), and only with *Msp*I did we obtain complete digestion exclusively for the strains of group B. Taken together, our data support the notion that *E. lentum* should be further differentiated by the sequence analysis of DNA in order to resolve the appropriate taxonomic status.

REFERENCES

1. **Brondz, I., and I. Olsen.** 1991. Multivariate analysis of cellular fatty acids in *Bacteroides*, *Prevotella*, *Porphyromonas*, *Wolinella*, and *Campylobacter* spp. J. Clin. Microbiol. **29:**183–189.
2. **Calhoon, D. A., W. R. Mayberry, and J. Slots.** 1981. Cellular fatty acid and soluble protein composition of *Actinobacillus actinomycetemcomitans* and related organisms. J. Clin. Microbiol. **14:**376–382.
3. **Chow, A. W., V. Patten, and L. B. Guze.** 1975. Susceptibility of anaerobic bacteria to metronidazole: relative resistance of non-spore-forming gram positive bacilli. J. Infect. Dis. **131:**182–185.
4. **Ghanem, F. M., A. C. Ridpath, W. E. C. Moore, and L. V. H. Moore.** 1991. Identification of *Clostridium botulinum*, *Clostridium argentinense*, and related organisms by cellular fatty acid analysis. J. Clin. Microbiol. **29:**1114–1124.
5. **Hewlett-Packard.** 1987. Hewlett-Packard 5898A Microbial Identification System operating manual. Version 3.0. Hewlett-Packard, Avondale, Pa.
6. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
7. **MacDonald, I. A., J. F. Jellett, D. E. Mahony, and L. V. Holdeman.** 1979. Bile salt 3α- and 12α-hydroxysteroid dehydrogenases from *Eubacterium lentum* and related organisms. Appl. Environ. Microbiol. **37:**992–1000.
8. **Moore, W. E., E. P. Cato, and L. V. Holdeman.** 1971. *Eubacterium lentum* (Eggerth) Prevot 1938: emendation of description and designation of the neotype strain. Int. J. Syst. Bacteriol. **21:**299–303.
9. **Mosca, A., C. A. Strong, and S. M. Finegold.** 1993. UV red fluorescence of *Eubacterium lentum*. J. Clin. Microbiol. **31:**1001–1002.
10. **National Committee for Clinical Laboratory Standards.** 1993. Reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Owen, R. J.** 1984. Nucleic acid sequencing and fingerprinting in bacterial classification, p. 21–31. *In* A. Sanna and G. Morace (ed.), New horizons in microbiology. Elsevier Science Publishing, Amsterdam, The Netherlands.
12. **Stoakes, L., T. Kelly, B. Schieven, D. Harley, M. Ramos, R. Lannigan, D. Groves, and Z. Hussain.** 1991. Gas-liquid chromatographic analysis of cellular fatty acids for identification of gram-negative anaerobic bacilli. J. Clin. Microbiol. **29:**2636–2638.
13. **Summanen, P., E. J. Baron, D. M. Citron, C. A. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing, Belmont, Calif.
14. **Verhulst, A., H. VanHespen, F. Symons, and H. Eyssen.** 1987. Systematic analysis of long chain components of Eubacterium lentum. J. Gen. Microbiol. **133:**275–282.

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