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Identification of *Campylobacter cinaedi* Isolated from Blood and Feces of Children and Adult Females

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Five *Campylobacter*-like organisms isolated from blood and feces were identified by numerical analysis of gel electrophoretic protein profiles and immunotyping as *Campylobacter cinaedi*. Two of these strains were isolated from adult females; the remaining three strains were isolated from children, two of whom were girls. *C. cinaedi* has hitherto been isolated only from rectal swabs and blood of homosexual and bisexual males with gastrointestinal symptoms. The results presented extend our knowledge of the features and the habitat of *C. cinaedi*.

In the past decade, the recognition of the clinical importance of campylobacters as gastrointestinal pathogens increased exponentially. Recently, a number of fastidious *Campylobacter*-like organisms (CLOs) were detected in rectal swabs from homosexual males (6). These organisms could be subdivided in three major DNA homology groups. Two of these groups were subsequently named *Campylobacter cinaedi* and *Campylobacter fennelliae* (33). The third group consisted of only one strain, which was referred to as the CLO-3 strain (33). *C. cinaedi* was reported to cause fever and bacteremia in homosexual men (3, 20, 23), and a successive bacteremia with *C. cinaedi* and *C. fennelliae* in a bisexual man was described by Ng et al. (20). The epidemiology of these infections remains unexplained; the only natural reservoir of *C. cinaedi* found so far is the normal intestinal tract of hamsters (10). Another group of fastidious CLOs was found in gastric biopsy specimens and was subsequently designated *Campylobacter pylori* (17, 18). These organisms may play an important role in the etiology of human gastritis (16, 18). A similar group of organisms was isolated from the gastric mucosae of ferrets and named *Campylobacter mustelae* (8, 9). The last two species were recently included in a new genus, *Helicobacter*, as *Helicobacter pylori* and *Helicobacter mustelae* (11). Fusiform bacteria biochemically resembling campylobacters were isolated from diarrheic stools (1) and from aborted ovine fetuses (2, 13) and were tentatively named "*Flexispira rappini*" (J. H. Bryner, J. Littleton, C. Gates, C. A. Kirkbride, A. E. Ritchie, and J. R. Archer, Proc. XIV Int. Congr. Microbiol., abstr. no. P.G11–18, p. 307, 1986). Thompson et al. (32) were the first to report that *C. pylori*, *C. cinaedi*, and *C. fennelliae* belong to a single rRNA homology group which should be removed from the genus *Campylobacter*. Furthermore, unpublished DNA-rRNA hybridization results of P. Vandamme and J. De Ley (manuscript in preparation) revealed that *H. mustelae*, CLO-3, and "*F. rappini*" also belong to the same rRNA homology group.

Immunotyping results pointed to a close relationship between *C. cinaedi* and five fastidious CLO strains isolated from blood and feces of children and adult females. Representative strains of *C. cinaedi*, *C. fennelliae*, *H. pylori*, *H. mustelae*, CLO-3, and "*F. rappini*" were included as references in the identification study of the five CLO strains. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins, followed by computer-assisted numerical analysis of the electrophoregrams, and immunotyping to characterize these strains.

MATERIALS AND METHODS

Strains used and growth conditions. The strains used and their sources are listed in Table 1. Names in quotation marks have not been validly published or included on the *Approved Lists of Bacterial Names*. In one culture (*H. mustelae* CCUG 23653), we found two colony types, which we designated t1 (large type) and t2 (small, pinpoint type). Both types were included in the SDS-PAGE study and displayed nearly identical protein electrophoregrams (Fig. 1). All strains were grown at 37°C on Mueller-Hinton agar (Oxoid CM337; Oxoid Ltd., Basingstoke, United Kingdom) supplemented with 5% (vol/vol) horse blood and incubated in a microaerophilic atmosphere consisting of approximately 4% CO_2, 5% O_2, 7.5% H_2, and 83.5% N_2 (obtained by replacing 75% of the air of an anaerobic jar without catalyst with a gas mixture containing 5% CO_2, 10% H_2, and 85% N_2).

PAGE of whole-cell proteins. All strains were grown on three to five petri dishes as described above. Whole-cell protein extracts were prepared, and SDS-PAGE was performed by a slightly modified version of the procedure of Laemmli (14) as described previously (34).

Numerical analysis of the protein gel electrophoregrams. The densitometric analysis, normalization, and interpolation of the protein gel electrophoregrams were performed as described by Pot et al. (26). Numerical analysis was performed as described by Kersters and De Ley (12) on points 1 to 350 of each interpolated trace. The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient r, and clustering was performed by the unweighted pair group method with average linkage (29).

Immunotyping analysis. Preparation of antigen, immunization, and immunodiffusion were performed by the method of Falsen (5) as described previously (34). Antisera were prepared against two CLO strains (CCUG 15432 and CCUG 17733), CLO-3 strain CCUG 14564, "*F. rappini*" CCUG 23435, and *H. pylori* CCUG 17874^T (superscript T indicates type strain) and CCUG 15816. Antigens against all CLO

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TABLE 1. Bacterial strains, strain numbers, and origins

Strain[a,b]	Other strain designation(s)[a,b]	Donor	Source	Place and yr of isolation
CLO strains				
CCUG 15432	LMG 8559	B. Claesson	Blood of a 42-yr-old woman	Skövde, Sweden (1984)
CCUG 17733	LMG 8558	Enteric Department (CCUG)	Feces of a 1-yr-old girl	Göteborg, Sweden (1985)
CCUG 19503	LCDC 86-8853, LMG 9357	H. Lior	Blood of a 24-yr-old woman with suppurative arthritis of the hip	Ottawa, Canada (1986)
CCUG 19504	LCDC 86-9267, LMG 9072	H. Lior	Blood of a 2-yr-old child with monoarticular arthritis	Ottawa, Canada (1986)
CCUG 20698	9258/86, LMG 9153	F. J. Bolton	Feces of a girl with diarrhea	Preston, United Kingdom (1987)
C. cinaedi CCUG 18818^T	165^T, LMG 7543^T	C. L. Fennell	Rectal swab from a homosexual man	Seattle, Wash. (1980–1983)
C. cinaedi CCUG 18819	929, LMG 9071	C. L. Fennell	Blood of a homosexual man	Houston, Tex.
C. cinaedi CCUG 19218	Goodwin 14446, LMG 8770	C. S. Goodwin	Rectal swab from a homosexual man	Seattle, Wash. (1980–1983)
C. fennelliae CCUG 18820^T	231^T, LMG 7546^T	C. L. Fennell	Rectal swab from a homosexual man	Seattle, Wash. (1980–1983)
C. fennelliae CCUG 19561	34349F/86, LMG 8771	F. J. Bolton	Feces of a healthy adult dog	Preston, United Kingdom (1986)
CLO-3 CCUG 14564	912/79, LMG 7792	M. B. Skirrow	Rectal swab from a homosexual man	Seattle, Wash. (1980–1983)
H. pylori CCUG 17874^T	KOO45659^T, LMG 7539^T	C. S. Goodwin	Endoscopic biopsy specimen of gastric mucosa	Perth, Australia (1982)
H. pylori CCUG 15816[c]	CT1, LMG 8773	B. J. Marshall	Duodenum	Perth, Australia (1984)
H. pylori CCUG 15818[c]	CT13, LMG 8927	B. J. Marshall	Gastric mucosa of a 65-yr-old man with a duodenal ulcer	Perth, Australia (1983)
H. pylori CCUG 19106	Pylo 10, LMG 8775	F. Mégraud	Gastric mucosa	Bordeaux, France (1986)
H. mustelae CCUG 23652	NCTC 12031, LMG 8776, LMG 8928	NCTC	Ferret gastric mucosa	Leeds, United Kingdom (1987)
H. mustelae CCUG 23653t1[d]	NCTC 12032, LMG 8777t1	NCTC	Ferret gastric mucosa	Leeds, United Kingdom (1987)
H. mustelae CCUG 23653t2[d]	NCTC 12032, LMG 8777t2	NCTC	Ferret gastric mucosa	Leeds, United Kingdom (1987)
"*F. rappini*" CCUG 23435	ATCC 43879, LMG 8738	ATCC	Patient with gastroenteritis	United States

[a] Type strains indicated by superscript T.

[b] ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; LMG, Culture Collection, Laboratorium voor Microbiologie, Rijksuniversiteit Gent, Ghent, Belgium; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London NW9 5HT, United Kingdom; F. J. Bolton, Public Health Laboratory Service, Preston Infirmary, Preston, United Kingdom; B. Claesson, Laboratory for Clinical Bacteriology, Skövde, Sweden; C. L. Fennell, Department of Medicine, Harborview Medical Center, Seattle, Wash.; C. S. Goodwin, Department of Microbiology, Royal Perth Hospital, Perth, Western Australia, Australia; H. Lior, National Enteric Reference Center, Laboratory Center for Disease Control (LCDC), Ottawa, Ontario, Canada; B. J. Marshall, Fremantle, Western Australia, Australia; F. Mégraud, Hôpital des Enfants, Centre Hospitalier Régional, Bordeaux, France; C. Patton, Centers for Disease Control, Atlanta, Ga.; M. B. Skirrow, Public Health Laboratory, Worcester, United Kingdom.

[c] Strains included in immunotyping analysis only.

[d] Strains included in SDS-PAGE only.

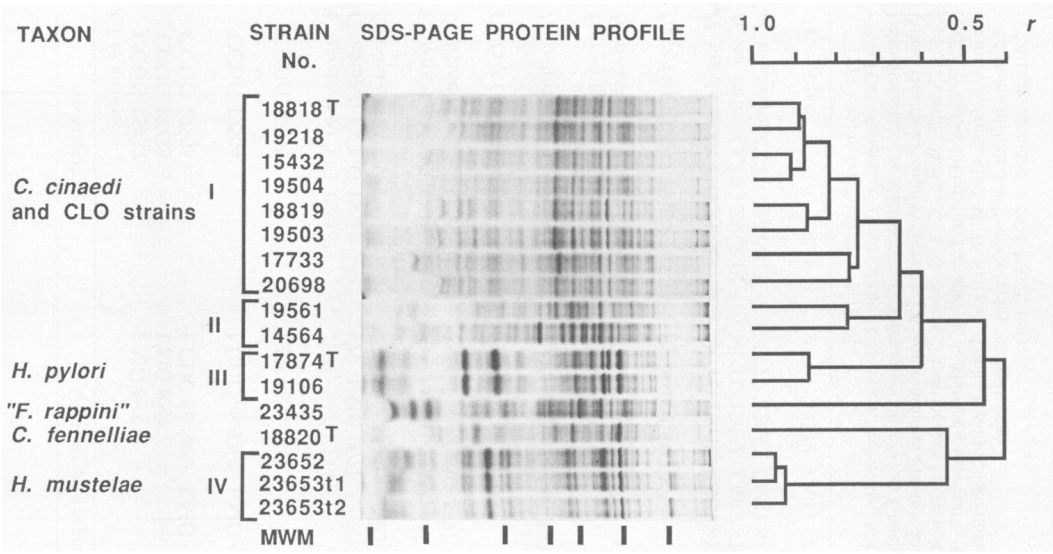

FIG. 1. Electrophoretic protein patterns and dendrogram derived from the unweighted pair group average linkage (29) of correlation coefficients (r) between the protein electrophoregrams of all CLO strains and *Campylobacter* reference strains. Roman numerals indicate cluster numbers. All strain numbers are CCUG (Culture Collection of the University of Göteborg, Göteborg, Sweden) numbers. T indicates type strain. The positions of the molecular weight markers (MWM) are indicated from left to right: lysozyme, molecular weight of 14,500; trypsin inhibitor, 20,100; carbonic anhydrase, 29,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; egg albumin, 45,000; bovine albumin, 66,000; and β-galactosidase, 116,000.

strains and all reference strains, with the exception of *H. mustelae* CCUG 23653, were prepared and cross-reacted with each antiserum.

RESULTS

PAGE of whole-cell proteins. As in other studies, we found an excellent agreement between the electrophoretic groupings after numerical analysis of the protein gel electrophoregrams and the named groups included (4, 30, 34) (Fig. 1). Duplicate protein extracts of several strains were prepared to verify the reproducibility of the growth conditions and the preparation of the extracts; a correlation level of $r \geq 0.92$ was found.

The numerical analysis revealed four clusters and two separate strains ("*F. rappini*" CCUG 23435 and *C. fennelliae* CCUG 18820^T) above the correlation level $r = 0.74$ (Fig. 1). Cluster I comprises the three *C. cinaedi* reference strains (including the type strain) and the five CLO strains grouping above $r = 0.75$. Cluster II consists of *C. fennelliae* CCUG 19561 and CLO-3 strain CCUG 14564, linked at $r = 0.76$, although the overall protein profiles of these strains are rather different. Cluster III, at $r = 0.86$, comprises the *H. pylori* strains (including the type strain), and cluster IV consists of the *H. mustelae* strains ($r > 0.93$).

Immunotyping. Six antisera and 16 antigens representing all taxa included in the SDS-PAGE study were cross-reacted with each other (Table 2). The five CLO strains and the *C. cinaedi* reference strains reacted in similar ways against each antiserum. Strong cross-reactions were found against the antiserum of CLO strain CCUG 15432. Somewhat weaker, but still clear-cut, cross-reactions against the antiserum of CLO strain CCUG 17733 were found; these cross-reactions were similar to the homologous precipitation reaction, indicating a high level of relatedness between *C. cinaedi* and the CLO strains. *H. pylori*, "*F. rappini*," and the CLO-3 strain showed strong cross-reactions with their homologous antiserum. The type strain of *C. fennelliae* CCUG 18820 showed a clear-cut but weak cross-reaction against the antiserum of CLO strain CCUG 15432. *C. fennelliae* CCUG 19561 did not cross-react with the antisera of the CLO strains CCUG 15432 and CCUG 17733; it showed, however, a strong cross-reaction against the antiserum of CLO-3 strain CCUG 14564 (Table 2).

All other cross-reactions revealed nonsignificant precipitation values.

DISCUSSION

We established the usefulness of SDS-PAGE and immunotyping for the identification of fastidious organisms in previous papers (28, 34). The application of gel electrophoresis of whole-cell proteins for identifying (4, 22, 30, 34) and typing campylobacters (7, 21) is increasingly gaining interest. In this study, our protein-electrophoretic and immunotyping techniques allowed a reliable identification of five CLO strains as *C. cinaedi*.

***C. cinaedi* and CLO strains.** The protein patterns of the *C. cinaedi* strains and the five CLO strains are very similar, with some variation in the 35,000- to 36,400-molecular-weight region. Deleting this variable region from the numerical analysis resulted in a higher clustering of these strains ($r > 0.80$; results not shown). It has been shown before within the genus *Campylobacter* (34) and within several other genera (12) that such high correlation coefficients between SDS-PAGE protein patterns reflect genomic relationships at the species level. Furthermore, as the *C. cinaedi* reference strains and all five CLO strains also yielded nearly identical precipitation values in the immunotyping analysis (Table 2), our results indicate unambiguously that all five CLO strains belong to *C. cinaedi*.

Before this study, *C. cinaedi* had been isolated mainly from feces or rectal swabs from homosexual or bisexual men (6). However, several investigators reported on bacteremia with *C. cinaedi* in a similar patient group (3, 20, 23). Two of our CLO strains were isolated from female adults, while the

TABLE 2. Immunotyping analysis

Source of antigen[a]	Score[b] with antiserum against:					
	CLO CCUG 15432	CLO CCUG 17733	CLO-3 CCUG 14564	*H. pylori* CCUG 17874^T	*H. pylori* CCUG 15816	"*F. rappini*" CCUG 23435
CLO strains						
CCUG 15432	7	4	0	0	0	2
CCUG 17733	6	4	0	0	0	2
CCUG 19503	6	2	0	0	0	2
CCUG 19504	5	2	0	0	—	—
CCUG 20698	6	3	0	0	0	—
C. cinaedi CCUG 18818^T	6	3	0	0	0	1
C. cinaedi CCUG 18819	7	2	0	0	0	2
C. cinaedi CCUG 19218	7	3	0	0	0	2
C. fennelliae CCUG 18820^T	3	0	2	0	0	0
C. fennelliae CCUG 19561	1	0	5	0	0	1
CLO-3 CCUG 14564	2	1	6	0	0	0
H. pylori CCUG 17874^T	0	0	0	7	8	1
H. pylori CCUG 19106	0	0	0	3	4	1
H. pylori CCUG 15818	0	0	0	5	8	1
H. mustelae CCUG 23652	0	0	0	2	2	0
"*F. rappini*" CCUG 23435	0	0	0	0	0	6

[a] Type strains indicated by superscript T.

[b] 0, No precipitate; 1 and 2, weak reaction with uncertain interpretation; 3, weak reaction usually revealing some relatedness; 4 and 5, moderate reaction revealing relatedness or identity with unsatisfactory antigen or serum; 6, 7, and 8, strong reaction observed only with closely related strains; —, analysis not performed. Each value is the average of at least two immunodiffusion analyses.

remaining three strains were recovered from children (two of them being girls) (Table 1). Strain CCUG 15432 was isolated from the blood of a woman without gastrointestinal symptoms and without any record of sexual contact with homosexuals. Strains CCUG 19503 and CCUG 19504 were isolated from the blood of patients with arthritis. Two strains (CCUG 20698 and CCUG 17733) were isolated from stools of children; at least one of these stools was diarrheic. To our knowledge, only Tee and co-workers (31) reported on the isolation of *C. cinaedi* from a woman. This strain was isolated from the feces of a female missionary worker with acute diarrheal gastroenteritis; two other strains were isolated from the feces of male patients with similar symptoms. Only one of these was isolated from a homosexual male (31).

We believe that our results indicate that *C. cinaedi* infections occur more widely than assumed so far. *C. cinaedi* infections do not seem to be restricted to homosexual or bisexual males. To determine the real occurrence rate and clinical importance of these very fastidious organisms, we recommend that clinical laboratories introduce routine isolation procedures for these organisms. However, commercially available agar bases and gas-generating kits do not always provide optimal incubation conditions (6, 15, 25, 27). An atmosphere containing hydrogen is strongly recommended whenever problems for growing campylobacters are encountered.

***C. fennelliae* and CLO-3.** Flores et al. (7) included six *C. fennelliae* strains in an SDS-PAGE study and found protein patterns that were nearly identical to one another. These profiles had a characteristic major protein band with a molecular weight of 66,000. The overall protein profile of the type strain of *C. fennelliae*, as determined in our study, is very similar to the one described by Flores et al. (7); it also contains the characteristic major protein band at a molecular weight of 66,000 (Fig. 1). However, *C. fennelliae* CCUG 19561, which was isolated from canine feces, displayed a protein profile very different from that of the type strain of *C. fennelliae*, with no major protein band with a molecular weight of 66,000 being present (Fig. 1). Immunotyping confirmed that this strain is very atypical, as the procedure revealed a close relationship between this strain and the CLO-3 strain of Totten et al. (33). Although both strains group in electrophoretic cluster II at $r = 0.76$, the overall protein profiles of these strains are rather different (Fig. 1). The grouping of these two strains in one cluster is probably due to the similar positions of a number of dense protein bands throughout the protein profile. We conclude that this strain does not belong to *C. fennelliae*; DNA-DNA hybridizations should be performed to elucidate the relationship between both strains.

***H. pylori*, *H. mustelae*, and "*F. rappini*."** Representative strains of these taxa were included as references in our study. The protein profiles and the precipitation values clearly distinguish these taxa from one another and from the other taxa studied (Fig. 1; Table 2). Both *H. pylori* strains displayed very similar protein profiles, in harmony with published data (19, 21, 24). The protein profiles of the three *H. mustelae* strains are nearly identical (Fig. 1).

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