Characterization of the Type Strain of *Campylobacter coli*, CIP 70.80, by Plasmid Typing

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A 1.9-kb plasmid DNA fragment from the type strain of *Campylobacter coli*, CIP 70.80, was used as a probe to characterize this type strain, other *C. coli* type strains obtained from several culture collections, and other *C. coli* strains. A specific hybridization pattern was obtained, and this pattern can be used to identify, characterize, and follow up *C. coli* type strains in culture collections.

Proper characterization of microorganisms is very important for taxonomists when they are setting up and surveying culture collections. Type strains are given particular attention for they reflect the general characteristics of species. Workers frequently propose that a type strain should be replaced with a neotype strain (9) and also frequently reinterpret taxonomic positions (14). Recently, a proposal for a new family, the family *Campylobacteraceae*, was published by Vandamme and De Ley (12), and the taxonomy of the genera *Campylobacter*, *Arco-bacter*, and *Helicobacter* has also been reviewed recently (13). In addition, minimal standards, including standards involving molecular data, have been proposed for the characterization of new species belonging to the family *Campylobacteraceae* (11).

Campylobacter are gram-negative bacteria which can be important human and animal pathogens (8, 10). *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter fetus* are the most representative species and are frequently responsible for

human enteritis, septicemia, and abortion, and for animal enteritis, abortion, and sterility (4, 6, 15). *C. coli* CIP 70.80^T was isolated from pig feces in 1970 and later was designated the type strain of the species and distributed to other culture collections. The aim of this study was to characterize and identify type strain *C. coli* CIP 70.80 and its derivatives by using molecular techniques. We describe a way to characterize this strain molecularly by using plasmid typing. A control investigation at the genome level was performed by using field inversion gel electrophoresis (FIGE).

The strains used in this study (Table 1) were grown for 48 h on plates containing Columbia agar supplemented with 5% sheep blood. The plates (diameter, 9 cm) were incubated at 37°C in a jar with a microaerobic atmosphere (BBL Campy-Pak; Becton Dickinson, Cockeysville, Md.). The bacteria were harvested with TE 100 (100 mM Tris-HCl [pH 8], 100 mM EDTA) and washed twice in the same buffer (5). Plasmid

TABLE 1. *C. coli* strains used in this study and their characteristics

Strain	Plasmid content ^a	Hybridization ^b	Direct source in this study ^c	Derivation ^c	First isolation ^c
<i>C. coli</i> CIP 70.80 ^T	+	+	CIP		A. Florent strain 1407
<i>C. coli</i> ATCC 33559 ^T	+	+	ATCC	CIP 70.80 ^T	
<i>C. coli</i> CCUG 11283 ^T	+	+	CCUG	CIP 70.80 ^T	
<i>C. coli</i> DSM 4689 ^T	+	+	DSM	CIP 70.80 ^T	
<i>C. coli</i> JCM 2529 ^T	+	+	JCM	CIP 70.80 ^T	
<i>C. coli</i> LMG 8847 ^T	+	+	LMG	CIP 70.80 ^T	
" <i>C. coli</i> LMG 6440 ^T "	+	+	UB	LMG←CIP 70.80 ^T	
" <i>C. coli</i> CIP 70.80 ^T "	+	+	UB	CIP 70.80 ^T	
<i>C. coli</i> NCTC 11366 ^T	+	+	NCTC	CIP 70.80 ^T	
<i>C. coli</i> CIP 70.54	—	—	CIP		A. Florent strain 3949
<i>C. coli</i> CIP 70.77	—	—	CIP		A. Florent strain P2
<i>C. coli</i> CIP 70.78	—	—	CIP		A. Florent strain P3
<i>C. coli</i> CIP 70.79	—	—	CIP		A. Florent strain P6
<i>C. coli</i> CIP 70.81	+	+	CIP		A. Florent strain P9
<i>C. coli</i> CIP 71.5	+	—	CIP		A. Florent strain P875
<i>C. coli</i> CIP 103753 ^d	+	—	CIP	BM	HB
<i>C. coli</i> A 630 ^d	—	—	HUSP		HUSP

^a +, plasmid DNA present; —, plasmid DNA absent.

^b +, hybridization with the 1.9-kb DNA fragment; —, no hybridization with the 1.9-kb DNA fragment.

^c ATCC, American Type Culture Collection, Rockville, Md.; BM, bioMérieux, Marcy l'Etoile, France; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; HB, Hôpital Pellegrin, Bordeaux, France; HUSP, Hôpital Universitaire Saint-Pierre, Brussels, Belgium; JCM, Japan Collection of Microorganisms, Wako, Saitama, Japan; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium; NCTC, National Collection of Type Cultures, London, United Kingdom; UB, University of Berne, Berne, Switzerland.

^d Human clinical isolate.

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DNA was extracted by the rapid alkaline lysis method (1), and whole-cell DNAs were isolated by using the lysozyme-sodium dodecyl sulfate (SDS)-proteinase K method described by Fennell et al. (3). Whole-cell and plasmid DNAs were digested with restriction enzymes *Hind*III and *Dra*I by using the instructions of the manufacturer (Boehringer, Mannheim, Germany). The restriction fragments were separated by electrophoresing them on a 1.2% agarose gel with TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA), denatured, neutralized, and transferred to Hybond-N filters (Amersham, Arlington Heights, Ill.) by Southern blotting. The probe used for hybridization was the 1.9-kb *Hind*III DNA fragment obtained after restriction of *C. coli* CIP 70.80^T (T = type strain) plasmid DNA, electroelution from the electrophoresis gel, and cloning into vector pUC18 (7). For hybridization reactions, the probe was freed from the vector and labeled with [α -³²P]dCTP (Amersham International, Amersham, United Kingdom) by using a random priming kit (Boehringer). The filters were prehybridized for 1 h at 65°C in a buffer containing 6× SSC, 5× Denhardt's solution, 0.5% SDS, and 100 µg of denatured salmon sperm DNA per ml (1× SSC is 150 mM NaCl plus 15 mM sodium citrate [pH 7.0]; 1× Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin). Hybridization was carried out at 65°C for 18 h in fresh prehybridization buffer supplemented with the radiolabeled probe (10⁶ cpm/µg of probe). After hybridization and stringent washing (twice with 2× SSC at 65°C for 10 min, once with 2× SSC–0.1% SDS at 65°C for 30 min, and once with 0.1× SSC at 65°C for 10 min), the filters were briefly air dried and exposed to Kodak XAR5 film with intensifying screens at –70°C.

For FGE analyses, DNAs were extracted from *Campylobacter* strains and restricted with *Sma*I or *Sal*I as described by Chang and Taylor (2). FGE was performed with a 1% agarose gel in TBE buffer (0.045 M Tris-borate, 0.001 M EDTA) at 250 V and 4°C for 22 h by using a field inversion pulse controller (model PC750; Hoefer Scientific Instruments, San Francisco, Calif.). The initial pulse durations were 1.5 s in the forward direction and 0.5 s in the reverse direction with a ramp factor value of 0.5 (i.e., the final pulse durations were 18 and 6 s, respectively).

An analysis of the plasmid content of *C. coli* CIP 70.80^T revealed that this strain contains a 70-kb low-copy-number plasmid and two 3.3-kb high-copy-number cryptic plasmids (8a). Digestion of the whole-plasmid DNA with enzyme *Hind*III yielded two major DNA fragments, a 1.6-kb fragment and a 1.9-kb fragment, which originated from the two small cryptic plasmids (Fig. 1A). These two DNA fragments exhibit extensive homology, as revealed by a DNA sequence analysis (8a). In this study, we used the 1.9-kb fragment as a probe to characterize and/or differentiate *C. coli* CIP 70.80^T, its derivatives (i.e., *C. coli* type strains from other culture collections), and other *C. coli* strains. Type strain CIP 70.80 and all of its derivatives yielded the same restriction and hybridization pattern in which two main fragments (1.6 and 1.9 kb) strongly hybridized to the probe (Fig. 1). After longer exposure times (1 h) three additional, fainter bands appeared (data not shown). The pattern obtained for *C. coli* CIP 70.81 DNA had a band at 1.6 kb and a second band at 3 kb. Two of the three additional fainter bands also appeared in this strain after longer exposure times. None of the other *C. coli* DNAs hybridized to the probe. When *Dra*I was used to digest the DNAs, the same strain grouping was obtained (data not shown).

The identical patterns found for type strain *C. coli* CIP 70.80 and its derivatives obtained from other culture collections suggest that these strains have not diverged from the original type strain, although successive subcultures have been made. In

FIG. 1. Characterization of *C. coli* strains by plasmid typing: *Hind*III restriction fragment length polymorphism of plasmid DNAs obtained after gel electrophoresis and ethidium bromide staining (A) and Southern blot of whole-cell DNAs digested with *Hind*III, probed with the ³²P-labeled 1.9-kb plasmid DNA fragment from *C. coli* CIP 70.80^T, and autoradiographed for 15 min (B). Lane 1, *C. coli* CIP 70.81; lane 2, *C. coli* CIP 70.80^T; lane 3, *C. coli* ATCC 33559^T; lane 4, *C. coli* CCUG 11283^T; lane 5, *C. coli* DSM 4689^T; lane 6, *C. coli* JCM 2529^T; lane 7, *C. coli* LMG 8847^T; lane 8, "*C. coli* LMG 6440^T"; lane 9, "*C. coli* CIP 70.80^T"; lane 10, *C. coli* NCTC 11366^T; lane 11, *C. coli* CIP 70.80^T after subculturing for 400 generations; lane 12, *C. coli* CIP 103753; lane 13, *C. coli* CIP 71.5; lane M, 1-kb molecular ladder (Gibco BRL, Gaithersburg, Md.).

vitro subculturing of type strain CIP 70.80 for about 400 generations (assuming a generation time of 1 h) under nonselective conditions did not result in a loss of plasmids. On the other hand, although *C. coli* CIP 70.80^T and CIP 70.81 seem to be very closely related, as suggested by the high levels of hybridization pattern homology obtained with their plasmids, there is no doubt that they are distinct organisms (Fig. 1). Moreover, whole-cell or plasmid DNAs from the other *C. coli* strains tested, especially *C. coli* CIP 103753, which is used as a quality control strain for API products, did not hybridize to the 1.9-kb probe (Fig. 1B and Table 1). Whole-cell DNAs from 11 other *Campylobacter* strains (*C. jejuni* CIP 70.2^T, *Campylobacter lari* CIP 102722^T, a *Campylobacter upsaliensis* clinical isolate, *Campylobacter fetus* subsp. *fetus* CIP 53.95, *C. fetus* subsp. *venerealis* CIP 68.29^T, a *Campylobacter hyointestinalis* clinical isolate, *Campylobacter sputorum* biovar *sputorum* CIP 103749^T, *C. sputorum* biovar *bulbus* CIP 53.103^T, *C. sputorum* biovar *fecalis* CIP 12014, a *Campylobacter concisus* clinical isolate, and a *Campylobacter curvus* clinical isolate) and from four non-*Campylobacter* strains (*Arcobacter cryaerophilus* CIP 104014, *Helicobacter pylori* CIP 101260, *Escherichia coli* HB101, and *Salmonella enterica* subsp. *enterica* serotype Typhimurium strain C53) also failed to hybridize to the probe.

FIG. 2. FIGE analysis of *C. coli* DNAs restricted with *Sma*I (lanes 2 to 19) or *Sal*I (lanes 21 to 32). Lanes 1, 20, and 33, λ ladder PFG marker (New England Biolabs); lanes 2 and 21, *C. coli* CIP 70.80^T; lanes 3 and 22, *C. coli* ATCC 33559^T; lanes 4 and 23, *C. coli* CCUG 11283^T; lanes 5 and 24, *C. coli* DSM 4689^T; lanes 6 and 25, *C. coli* JCM 2529^T; lanes 7 and 26, *C. coli* LMG 8847^T; lanes 8 and 27, "*C. coli* LMG 6440^T"; lanes 9 and 28, "*C. coli* CIP 70.80^T"; lanes 10 and 29, *C. coli* NCTC 11366^T; lanes 11 and 30, *C. coli* CIP 70.80^T after subculturing for 400 generations; lanes 12 and 31, *C. coli* CIP 70.81; lanes 13 and 32, *C. coli* CIP 103753; lane 14, *C. coli* CIP 71.5; lane 15, *C. coli* CIP 70.79; lane 16, *C. coli* CIP 70.78; lane 17, *C. coli* CIP 70.77; lane 18, *C. coli* CIP 70.54; lane 19, *C. coli* A630.

FIGE analysis of the *C. coli* strains confirmed the genomic identity between type strain *C. coli* CIP 70.80 and its derivatives, whereas different patterns were obtained with *C. coli* CIP 70.81 as well as the other *C. coli* strains tested (Fig. 2).

Our results indicate that molecular typing in which the 1.9-kb plasmid DNA probe from *C. coli* CIP 70.80^T is used is a reliable method for characterizing the *C. coli* type strain and for studying its possible evolution.

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