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, Arcobacter, 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).

Campylobacters 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 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-Cl [pH 8.0], 100 mM EDTA) and washed twice in the same buffer (5).

Plasmid

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid contenta</th>
<th>Hybridizationb</th>
<th>Direct source in this studyc</th>
<th>Derivationd</th>
<th>First isolationd</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. coli CIP 70.80T</td>
<td>+</td>
<td>+</td>
<td>CIP</td>
<td>A. Florent strain 1407</td>
<td></td>
</tr>
<tr>
<td>C. coli ATCC 33559T</td>
<td>+</td>
<td>+</td>
<td>ATCC</td>
<td>CIP 70.80T</td>
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</tr>
<tr>
<td>C. coli CCUG 11293T</td>
<td>+</td>
<td>+</td>
<td>CCUG</td>
<td>CIP 70.80T</td>
<td></td>
</tr>
<tr>
<td>C. coli DSM 4689T</td>
<td>+</td>
<td>+</td>
<td>DSM</td>
<td>CIP 70.80T</td>
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</tr>
<tr>
<td>C. coli JCM 2529T</td>
<td>+</td>
<td>+</td>
<td>JCM</td>
<td>CIP 70.80T</td>
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<tr>
<td>C. coli LMG 8847T</td>
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<td>+</td>
<td>LMG</td>
<td>CIP 70.80T</td>
<td></td>
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<tr>
<td>“C. coli LMG 64407”</td>
<td>+</td>
<td>+</td>
<td>UB</td>
<td>LMGC–CIP 70.80T</td>
<td></td>
</tr>
<tr>
<td>“C. coli CIP 70.80”</td>
<td>+</td>
<td>+</td>
<td>UB</td>
<td>CIP 70.80</td>
<td></td>
</tr>
<tr>
<td>C. coli NCTC 11366T</td>
<td>+</td>
<td>+</td>
<td>NCTC</td>
<td>CIP 70.80T</td>
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<tr>
<td>C. coli CIP 70.54</td>
<td>–</td>
<td>–</td>
<td>CIP</td>
<td>A. Florent strain 3949</td>
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<td>–</td>
<td>CIP</td>
<td>A. Florent strain F2</td>
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<tr>
<td>C. coli CIP 70.79</td>
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<td>–</td>
<td>CIP</td>
<td>A. Florent strain F3</td>
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<td>C. coli CIP 70.81</td>
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<td>–</td>
<td>CIP</td>
<td>A. Florent strain P9</td>
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<tr>
<td>C. coli CIP 71.5</td>
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<td>–</td>
<td>CIP</td>
<td>A. Florent strain P875</td>
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<td>C. coli CIP 103753d</td>
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<td>–</td>
<td>CIP</td>
<td>HB</td>
<td></td>
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<tr>
<td>C. coli A 630T</td>
<td>+</td>
<td>–</td>
<td>HUSP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 C. coli ATCC American Type Culture Collection, Rockville, Md.; BM, bioMérieux, Marcy l’Etoile, France; CCUG, Culture Collection of the University of Goeteborg, Goeteborg, 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 Bern, Bern, Switzerland.
d Human clinical isolate.

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DNAs were extracted by the rapid alkaline lysis method (1), and whole-cell DNAs were isolated by using the lysozyme-sodium dodecyl sulfate (SDS)-protease K method described by Fennell et al. (3). Whole-cell and plasmid DNAs were digested with restriction enzymes HindIII and DraI 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 HindIII DNA fragment obtained after restriction of C. coli CIP 70.80\(^T\) (T = type strain) plasmid DNA, electrophoresion from the electrophoresis gel, and cloning into vector pUC18 (7). For hybridization reactions, the probe was freed from the vector and labeled with \([\alpha-^3\Pd]\)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 6X SSC, 5X Denhardt's solution, 0.5% SDS, and 100 µg of denatured salmon sperm DNA per ml (1X SSC is 150 mM NaCl plus 15 mM sodium citrate [pH 7.0]; 1X 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 (10\(^{6}\) cpm/µg of probe). After hybridization and stringent washing (twice with 2X SSC at 65°C for 10 min, once with 2X SSC–0.1% SDS at 65°C for 30 min, and once with 0.1X 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 FIGE analyses, DNAs were extracted from Campylobacter strains and restricted with Smal or Saff as described by Chang and Taylor (2). FIGE 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 HindIII 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 DraI was used to digest the DNAs, the same 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 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 70.81 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 upsuliensis clinical isolate, Campylobacter fetus subsp. fetus JCM 53.95, C. fetus subsp. vernerensis CIP 68.29\(^T\), a Campylobacter hyointestinalis clinical isolate, Campylobacter sp. sp. sp. H. pylori ATCC 43014, 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. 1. Characterization of C. coli strains by plasmid typing: HindIII 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 HindIII, 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 CIP 12014, Helicobacter pylori CIP 101260; lane 5, C. coli CIP 70.80; lane 6, C. coli JCM 53.95; 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 CIP 70.80\(^T\); 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.).]
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 is used is a reliable method for characterizing the C. coli type strain and for studying its possible evolution.

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REFERENCES