Available online at www.sciencedirect.com
ScienceDirect
Plasmid 57 (2007) 108–117
PLASMID
www.elsevier.com/locate/yplas

Cryptic plasmids isolated from *Campylobacter* strains represent multiple, novel incompatibility groups

William G. Miller *, Sekou Heath, Robert E. Mandrell

Produce Safety and Microbiology Research Unit, Agricultural Research Service, US Department of Agriculture, Albany, CA 94710, USA

Received 12 May 2006, revised 22 August 2006
Available online 24 October 2006
Communicated by Grezegorz Wegrzyn

Abstract

Three small, cryptic plasmids from the multi-drug-resistant (MDR) *Campylobacter coli* strain RM2228 and one small, cryptic plasmid from the MDR *Campylobacter jejuni* strain RM1170 were sequenced and characterized. pCC2228-1 has some similarity to Firmicutes RepL family plasmids that replicate via a rolling-circle mechanism. pCC2228-2 is a theta-replicating, iteron-containing plasmid (ICP) that is a member of the same incompatibility (Inc) group as previously described *Campylobacter* shuttle vectors. The other two ICPs, pCC2228-3 and pCJ1170, represent a second novel Inc group. Comparison of the four plasmids described in this study with other characterized plasmids from *C. jejuni*, *C. coli*, *C. lari*, and *C. hyointestinalis* suggests that cryptic plasmids in *Campylobacter* may be classified into as many as nine Inc groups. The plasmids characterized in this study have several unique features suitable for the construction of novel *Campylobacter* shuttle vectors, e.g., small size, absence of many common multiple-cloning site restriction sites, and Inc groups not represented by current *Campylobacter* shuttle plasmids. Thus, these plasmids may be used to construct a new generation of *Campylobacter* shuttle vectors that would permit transformation of environmental *Campylobacter* isolates with an existing repertoire of native plasmids.
Published by Elsevier Inc.

Keywords: *Campylobacter*; Plasmids; Incompatibility; Rolling-circle; Iteron; Copy number

1. Introduction

The Gram-negative bacterium *Campylobacter jejuni* is a major cause of human diarrheal disease. Many genetic tools have been developed for enterobacterial pathogens such as *Escherichia coli* and *Salmonella enterica*; however, these tools have not been used successfully in *Campylobacter*. For example, broad-host-range plasmids from a variety of incompatibility (Inc) groups exist and are a valuable resource for studies of many enterobacterial taxa, but they do not replicate in *Campylobacter* (Guerry et al., 1994). To overcome this difficulty, several series of shuttle plasmids that contain *E. coli* and *Campylobacter coli* origins of replication for effective use in *Campylobacter* have been constructed (Guerry et al., 1991; Labigne-Roussel et al., 1987; Miller et al., 2000; Wang and Taylor,

* Corresponding author. Fax: +1 510 559 6162.
E-mail address: bmiller@pw.usda.gov (W.G. Miller).

0147-619X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.plasmid.2006.08.005

1990a,b; Yao et al., 1993). However, these shuttle plasmids have three basic limitations: (1) with one exception, all *Campylobacter* shuttle plasmids are derived, in part, from the cryptic *C. coli* plasmid pIP1455 (Labigne-Roussel et al., 1987) and, therefore, are members of the same Inc group. Accordingly, these multiple pIP1455-derived shuttle plasmids can only co-exist over multiple generations in the same strain if each plasmid contains a different antibiotic-resistance marker and double selection is maintained. A shuttle plasmid was constructed from the *C. jejuni* cryptic plasmid pCJ419 (Alfredson and Korolik, 2003), but no data are available as to whether pCJ419 is in a different Inc group than pIP1455. (2) The *C. coli* origin of replication present in the shuttle vectors is rather large, resulting in an average plasmid size of 7 kb; overall plasmid size is important as *Campylobacter* shuttle plasmids containing large inserts are often unstable (data not shown). (3) Drug-resistance genes for only three antibiotics, kanamycin (Labigne-Roussel et al., 1987; Wang and Taylor, 1990b; Wosten et al., 1998; Yao et al., 1993), chloramphenicol (Wang and Taylor, 1990a; Yao et al., 1993), and tetracycline (Wang and Taylor, 1990b), are present on *Campylobacter* shuttle plasmids. Because several *Campylobacter* strains have a natural low-level resistance to kanamycin and some strains are resistant to chloramphenicol and/or tetracycline, additional drug-resistance genes would increase the utility of *Campylobacter* shuttle plasmids.

Small plasmids have been found in several *Campylobacter* species (Alfredson and Korolik, 2003; Jesse et al., 2006; Labigne-Roussel et al., 1987; Luo and Zhang, 2001; Waterman et al., 1993) and many plasmids (Trieber and Taylor, 2000) contain antibiotic-resistance genes. Therefore, it was reasonable to assume that additional antibiotic-resistance genes might be present on plasmids and an obvious source of such plasmids would be multi-drug-resistant (MDR) *Campylobacter* strains. The goal of this study was to characterize several small plasmids from MDR *Campylobacter* in order to solve one or more of the limitations described above.

2. Materials and methods

2.1. Plasmid sequencing and analysis

Two micrograms of undigested genomic DNA (strains RM2228 and RM1170) was run on a 1% agarose gel. Plasmid bands were excised from the gel and eluted. Purified plasmids were digested with *Hin*dIII and cloned into pUC18; the *Hin*dIII subclones were then sequenced using the standard M13 forward and reverse sequencing primers. The order of the *Hin*dIII fragments for each plasmid was determined by designing unique primers directed towards the ends of each fragment and performing pairwise amplifications. Each amplicon was then sequenced to fill in the sequence gaps between the *Hin*dIII fragments. Sequence gaps within the fragments were completed by primer walking.

Cycle sequencing reactions were performed on an Applied Biosystems 9700 thermocycler (Applied Biosystems, Foster City, CA) using the ABI PRISM BigDye terminator cycle sequencing kit (version 1.0 or version 3.0) and standard protocols as recommended by the manufacturer. All extension products were purified on Centri-Sep spin columns (Princeton Separations, Adelphia, NJ). DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using the POP-6 polymer and ABI PRISM Genetic Analyzer Data Collection and ABI PRISM Genetic Analyzer Sequencing Analysis software. Sequencing oligonucleotide primers were purchased from either Oligos Etc. (Wilsonville, OR) or Integrated DNA Technologies (Coralville, IA). Sequencing reads were trimmed and assembled using Lasergene (ver. 5.0; DNAstar, Madison, WI).

The nucleotide sequences of pCC2228-1, pCC2228-2, pCC2228-3, and pCJ1170 have been deposited in the GenBank database and assigned the following accession numbers: DQ518170, DQ518171, DQ518172, and DQ518173, respectively.

2.2. Plasmid copy number quantification

RM2228 plasmid copy number was determined by quantitative PCR (QPCR). Three QPCR primer pairs were designed for plasmid pCC2228-1, four for plasmid pCC2228-2, and four for plasmid pCC2228-3. Six QPCR primer pairs were designed to amplify six different chromosomal genes; these genes were located near the predicted terminus of replication to minimize replication effects on copy number and achieve an approximate copy number of one, as genes near the replication terminus often have a two- to threefold lower copy number relative to genes near the origin of replication, under many growth conditions (Miller and Simons, 1993; Pavitt and Higgins, 1993; Sousa et al., 1997). As an internal control, three pairs of QPCR primers were designed to amplify the ribosomal RNA loci.

All QPCRs were run on an MX3000P QPCR system (Stratagene, La Jolla, CA) and all analysis was performed on the accompanying software. Each reaction contained: 12.5 μl Brilliant SYBR Green QPCR master mix (Stratagene), 1 μl each primer (75 nM final concentration; MWG-Biotech, High Point, NC), 0.313–10 ng template DNA and ddH_2O to volume (25 μl). Template DNA was prepared by resuspending approx. 10 μl RM2228 cells,