Plasmid 70 (2013) 268–271

Contents lists available at SciVerse ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Short Communication

Characterization of the *Campylobacter jejuni* cryptic plasmid pTIW94 recovered from wild birds in the southeastern United States

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ARTICLE INFO

Article history:
Received 28 November 2012
Accepted 17 April 2013
Available online 30 April 2013
Communicated by David Keith Summers

Keywords:
Cryptic plasmid
Campylobacter spp.
Birds

ABSTRACT

The complete nucleotide sequence was determined for a cryptic plasmid, pTIW94, recovered from several *Campylobacter jejuni* isolates from wild birds in the southeastern United States. pTIW94 is a circular molecule of 3860 nucleotides, with a G + C content (31.0%) similar to that of many *Campylobacter* spp. genomes. A typical origin of replication, with iteron sequences, was identified upstream of DNA sequences that demonstrated similarity to replication initiation proteins. A total of five open reading frames (ORFs) were identified; two of the five ORFs demonstrated significant similarity to plasmid pCC2228-2 found within *Campylobacter coli*. These two ORFs were similar to essential replication proteins RepA (100%; 26/26 aa identity) and RepB (95%; 327/346 aa identity). A third identified ORF demonstrated significant similarity (99%; 421/424 aa identity) to the MOB protein from *C. coli* 67–8, originally recovered from swine. The other two identified ORFs were either similar to hypothetical proteins from other *Campylobacter* spp., or exhibited no significant similarity to any DNA or protein sequence in the GenBank database. Promoter regions (−35 and −10 signal sites), ribosomal binding sites upstream of ORFs, and stem-loop structures were also identified within the plasmid. These results demonstrate that pTIW94 represents a previously un-reported small cryptic plasmid with unique sequences as well as highly similar sequences to other small plasmids found within *Campylobacter* spp., and that this cryptic plasmid is present among *Campylobacter* spp. recovered from different genera of wild birds.

1. Introduction

Campylobacter jejuni and *Campylobacter coli*, Gram-negative, microaerophilic bacteria, are currently responsible for approximately 17.1% of all domestically acquired foodborne related hospitalizations in the United States (Scallan et al., 2011). Most human foodborne *Campylobacter* spp. infections are associated with the consumption of poultry or poultry products (Friedman et al., 2004; Nielsen et al., 2006), even though the organism is usually not clinically important for birds (Shane, 2000). The majority of *C. jejuni* human cases are enteric, with most episodes confined to local acute gastroenteritis characterized by nausea, abdominal cramps, diarrhea, and fatigue. Infections are generally self-limited and are resolved within several days after initial onset. *Campylobacter* spp. infections have also been associated with unnecessary appendectomies, reactive arthritis, and the development of Guillain–Barré

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0147-619X/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.plasmid.2013.04.004

syndrome, although these complications are infrequent (Bokkenheuser and Sutter, 1981; Bryan and Doyle, 1995; Butzler and Skirrow, 1979; Walker et al., 1986).

Several investigations focused on delineating the epidemiology of *Campylobacter* spp. have been conducted; however, a complete understanding of the critical sources for *Campylobacter* spp. transmission remains elusive. Additionally, as the interface between human and wildlife habitats contract, the potential for emerging vectors for transmission arise (Humphrey et al., 2007). One vector postulated to be involved in the transmission of zoonotic pathogens, such as *Campylobacter* spp., is the wild bird. Migratory and other wild birds can disseminate potential zoonotic pathogens such as West Nile virus, Avian influenza and enteric bacteria, as well as antimicrobial resistance genes (Broman et al., 2002; Colles et al., 2009; Dolejska et al., 2007; Gaidet et al., 2012; Ganapathy et al., 2007; Keller et al., 2011; Reed et al., 2003; Robino et al., 2010; Waldenstrom et al., 2005). Additionally, surface waters that may be subsequently used for drinking, recreation, or crop irrigation (especially the irrigation of fresh produce), were contaminated with relatively high levels of enteric bacteria (Duffy, 2003; Jokinen et al., 2011; Kinzelman et al., 2008). Given the observation that numerous *Campylobacter* spp. are known to possess small plasmids (Alfredson and Korolik, 2003; Jesse et al., 2006; Luo and Zhang, 2001; Miller et al., 2007), many of which contain antibiotic resistance genes (Trieber and Taylor, 2000), and that migratory birds are a significant source for dissemination of *Campylobacter* spp. to the human population, the characterization of plasmids within wild bird populations is of importance to public health. Therefore, we characterized a small cryptic plasmid, from *C. jejuni*, recovered during a survey of wild or migratory birds conducted in the southeastern United States.

2. Materials and methods

2.1. Geographical locations, sampling, and microbiological procedures

C. jejuni isolates were recovered during a convenience sampling (cloacal swabs) of wild birds at landfills located in the southeastern United States. Samples were homogenized, using a Pulsifier (Microbiology International, Fredrick, MD), and tested for *Campylobacter* spp. using the Cape Town Recovery method (Lastovica and Le Roux, 2001) (personal communication with members of the EC FP5-CAMPYCHECK project QLK CT 2002 02201). Briefly, a 50 mm, 0.6 μm mixed cellulose ester filter (Whatman, Schleicher & Schuell; Dassel, Germany) was aseptically placed in the center of a Brucella agar plate supplemented with *Campylobacter* spp. growth supplement SR84™ (Oxoid/Remel, Lenexa, KS) and 10% laked horse blood (Oxoid/Remel). Aliquots of homogenized fecal sample were applied to the filter and allowed to set, covered, at room temperature for 15 min. The filter was aseptically removed, using sterile tweezers, and incubated at 37 °C in a hydrogen enriched atmosphere (7.5% H_2, 2.5% O_2, 10% CO_2, and 80% N_2).

2.2. Plasmid DNA isolation, cloning and DNA sequence analyses

Plasmid DNA, recovered from *C. jejuni* isolates, was isolated using the QIAprep™ Miniprep kit (Qiagen, Valencia, CA). The isolated plasmids were restricted with *Hin*dIII (New England BioLabs, Beverly, MA) followed by cloning of resulting bands into the pGEM®-3Z™ vector (Promega Corporation, Madison, WI). Ligation reactions were subsequently transformed into *Escherichia coli* strain DH5αmcr⁻™ chemically competent cells (Invitrogen, Carlsbad, CA), plated onto LB agar plates supplemented with 50 μg/ml ampicillin, and incubated overnight at 37 °C. Resulting white transformants were picked, transferred to LB broth supplemented with ampicillin (100 μg/ml), and grown at 37 °C overnight with agitation (200 rpm). Initial DNA sequence data was generated using the M13Forward or M13Reverse primers with the Big-Dye Dye-Terminator Cycle Sequencing Kit (ABI-PE, Foster City, CA). Data was assembled with Sequencher 5.1 (GeneCodes Corp., Ann Arbor, MI) and aligned using ClustalX. Subsequent sequence data was generated by primer walking. Primers were designed using the PrimerSelect Program from the Lasergene Software Suite (DNA*, Madison, WI) and are listed in Table 1. Open reading frames were identified using both ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/) and GeneMark.hmm for Prokaryotes, Version: 2.4 (http://exon.biology.gatech.edu/). The complete sequence of the pTIW94 plasmid was deposited in GenBank under Accession No. BankIt1632039 pTIW94 KF192842.

3. Results and discussion

Genomic DNA recovered from isolates of *C. jejuni* originating from a European starling (*Sturnus vulgaris*) and four crows (*Corvus* spp.), sampled from a landfill, revealed a

Table 1
Primer sequences used for primer walking analyses of pTIW94.

Primer name	Sequence (5′-3′)
CC1	AGC CCC TTG CCT TGA TTG TC
	TGC ATA TCC AAA GTA AGA CAA TC
CC2	CTG TTA GTT CCG TTC CCC GA
	CTA ACA GAA TGA AAC CTA TAC GT
CC3	AAA ATA ATG AGC GAT GAG CAA AAA
	ATT TTT GCT CAT CGC TCA TTA TTT
CC4	AAA AAC GAG TAG CGA GTA ATA AAA
	TTT ATT ACT CGC TAC TCG TTT TTA
CC5	TGC AAA AGC TAG AAA ATG AAA GTC
	TAT TTT TGC TCA TCG CTC ATT ATT
CC6	CTG AAA GTA AAA GAT CGA AAA CGT
	TTA TTA CTC GCT ACT CGT TTT TAT
CC7	AAT GCA AAA ACA ATA TCC AGA AGT
	ATG CAC TTC TGG ATA TTG TTT TTG
CC8	TGA AGA CCT ATA ACA AAA ACG TAA
	GTT TTT GTT ATA GGT CTT CAC GTA
CC9	TAG TAA CGG CCG CCA GTG TGC
	AAG GGC GAA TTC CAG CAC ACT
CC10	CGT GTG ACC GCC GGC AAT GAT
	TCA CAC GAC CTT AAG CGG GAA
CC11	TTA TCT TAG GTT TAG CAG TTA TTG
	TAA GGC AAC GCT AGA TAA TGA A
CC12	GTT ATT GAC GAT TTG GAT TCT TTA
	AAG TAA TAG ATC GCA ACG GAA T