Indian J Microbiol (Jan–Mar 2014) 54(1):33–40
DOI 10.1007/s12088-013-0406-z

ORIGINAL ARTICLE

Identification and Characterization of an Invasion Antigen B Gene from the Oral Pathogen *Campylobacter rectus*

Michael J. LaGier · Deborah S. Threadgill

Received: 14 August 2012 / Accepted: 12 April 2013 / Published online: 21 April 2013
© Association of Microbiologists of India 2013

Abstract The oral bacterium, *Campylobacter rectus*, is an etiological agent of periodontitis. The virulence genes of *C. rectus* are largely unknown. The aim of this study was to query *C. rectus* for the presence of an invasion antigen B (*ciaB*) gene, which is needed for cell invasion by the related species *Campylobacter jejuni*. PCR and PCR-walking identified a *ciaB* from *C. rectus*. In silico analyses of *C. rectus* 314 *ciaB* (*Cr-ciaB*) revealed an ORF of 1,830 base pairs. The Cr-CiaB protein shared significant sequence identity (BLASTx and phylogeny) with CiaB from related campylobacters. Cr-CiaB is predicted to lack membrane helices, signal peptides, and localizes to the cytoplasm; which are consistent with CiaB proteins. Expression of *Cr-ciaB* was confirmed with RT-PCR; and potential *ciaB* genes were detected in eight additional strains of *C. rectus*. *Cr-ciaB* is the first CiaB identified from the oral campylobacters.

Keywords *Campylobacter rectus* · Periodontitis · Invasion antigen B · Virulence factor

Introduction

Periodontitis is an inflammatory disorder of connective tissue and bone that support the teeth [1]. Periodontitis is common, affecting 35 % of adults [1, 2]. Approximately 13 % of patients develop severe forms of periodontitis, which, if untreated, may result in tooth loss and systemic complications including an increased risk of heart attack and an increased risk of pregnancy complications [1, 2].

Campylobacter rectus is a poorly described Gram-negative, oral bacterium that has been implicated as a cause of periodontitis [3–5]. Recent studies indicate that women with periodontitis are seven times more likely to experience preterm labor than their healthy counterparts [6, 7]. Serological data from human studies has implied that *C. rectus*, as part of the poly-microbial oral community, plays a role in the preterm labors of mothers with periodontitis [8]. Additionally, a pregnant mouse model has shown the association of *C. rectus* strain 314, isolated from a patient with periodontitis [9], with fetal growth restriction and a decreased survival of pups [10, 11]. Although the aforementioned human and mouse studies have established *C. rectus* as an agent of periodontitis, the pathogenic mechanisms of *C. rectus* are not well elucidated.

Although *C. rectus* strain 33238 has been partially sequenced (NCBI GenBank #ACFU00000000), the genes important to the virulence and pathogenic mechanisms of *C. rectus* have not been identified. To understand *C. rectus* as an oral pathogen, querying multiple strains for the presence of virulence factors may be significant as strain-to-strain genomic variability has been seen among campylobacters [12, 13]. Recently, a few studies have identified potential virulence factors in *C. rectus*, including potential toxin genes (*csx A* and *B; csxC and csxD*) and a surface array protein (*crsA* gene) thought to play a role in allowing the bacteria to avoid the host immune system [14–16]. While these studies demonstrate that *C. rectus* encodes potential virulence genes; it is unknown whether *C. rectus* contains genes that have been shown to significantly

M. J. LaGier (✉)
Department of Biological Sciences, Florida Gulf Coast University, 10501 FGCU Boulevard South, Fort Myers, FL 33965, USA
e-mail: mlagier@fgcu.edu

D. S. Threadgill
Department of Microbiology, North Carolina State University, Raleigh, NC, USA

contribute to the pathogenesis of related campylobacters, including ciaB.

Within the campylobacters, virulence factors have been best characterized from the gastrointestinal pathogen *Campylobacter jejuni* [17]. Among the best studied *C. jejuni* virulence factors is the *ciaB* gene, which has been shown to play a critical role in host cell invasion; and therefore pathogenesis [18–20]. In addition, *ciaB* genes have been identified from additional campylobacters including *Campylobacter lari*, *Campylobacter coli*, and *Campylobacter upsaliensis* [21, 22]. Given the conservation of *ciaB*, and a recent study showing *C. rectus* 314 can invade host cells [23], we hypothesized that the *C. rectus* 314 contains a *ciaB* gene. In this study, a combination of PCR, PCR-walking, reverse transcriptase PCR, and in silico tools were used to characterize a *ciaB* gene (*Cr-ciaB*) from *C. rectus* 314. The identification of *C. rectus* CiaB suggests that CiaB might contribute to the pathogenesis of *C. rectus* during host cell invasion [23]; and raises the possibility of developing novel periodontal therapeutics that disrupt CiaB functionality.

Materials and Methods

Bacterial Strains and Genomic DNA Isolation

Campylobacter curvus (ATCC 33273) and the *C. rectus* strains listed in Table 1 were grown under standard anaerobic conditions. *C. curvus,* like *C. rectus*, is an oral bacteria associated with periodontitis [36]. Bacteria were grown on tryptic soy blood agar with sodium formate (0.3 %). Genomic DNA was isolated from bacterial pellets using CTAB [24].

Table 1 Summary of *Campylobacter rectus* strains used in this study

Strain	Description
33238	ATCC type strain (http://www.atcc.org)
314	Periodontal isolate [9]
CCUG 11645	Periodontal isolate[a]
CCUG 11643	Periodontal isolate[a]
CCUG 11642	Periodontal isolate[a]
CCUG 11640	Periodontal isolate[a]
CCUG 27948	Periodontal isolate[a]
CCUG 48803	Periodontal isolate[a]
BCCM 7615	Periodontal isolate[b]

[a] University of Göteborg, Sweden (http://www.ccug.se)

[b] Belgian collection of microorganisms (http://bccm.belspo.be/index.php)

Degenerate PCR

Genomic DNA from *C. rectus* 314 and 33238 were scanned for *ciaB* genes using oligonucleotides CiaB-DF and CiaB-DR (Table 2). CiaB-DF and CiaB-DR were designed to amplify conserved regions within known *ciaB* [25]. PCR was then used to screen *C. rectus* for the presence of *ciaB*. Each 50 µl PCR reaction contained 1.0 µM of each primer. The cycling conditions used were as follows: 94 °C for 1 min, 40 °C for 1 min, and 72 °C for 1 min (10 cycles); followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. Amplicons were purified (QIAquick gel extraction kit, Qiagen, USA) and cloned for sequencing into pCR2.1-TOPO (TOPO-TA kit, Invitrogen, USA).

RNA Isolation and Reverse Transcription PCR

RNA was isolated from bacterial pellets using a QIAgen miniprep kit (Qiagen, USA). The RNA was then used for RT-PCR using the Access RT-PCR kit (Promega, USA).

PCR-Walking

A Genome Walker kit (Clontech, USA) was used to obtain the ORF of *ciaB*. Genome Walker is a PCR method for identifying sequences adjacent to known sequences. The kit was used according to the manufacturer's protocol. In brief, the sequences identified by PCR were used to design gene-specific primers (Table 2). Next, 2.5 µg of 314 genomic DNA was digested with one of four blunt-ended restriction endonucleases: *DraI*, *EcoRV*, *PvuII* or *StuI*. The digested DNA reactions was then ligated to adapter oligonucleotides. The ligated products were then used as templates for PCR using primers complementary to the adapters (Table 2, AP1) and complementary to *ciaB* (Table 2, CiaB-Walk-5R or CiaB-Walk-3F). A primer combination of AP1 and CiaB-Walk-5R was used to obtain the 5′-end of *ciaB*; while AP1 and CiaB-Walk-3R was used to obtain the 3′-end of *ciaB*. PCR products were purified (QIAquick gel extraction kit) and cloned for sequencing into pCR2.1-TOPO.

Sequence Analysis

DNA chromatographs were edited manually and assembled using Vector NTI software (Invitrogen, USA). Obtained sequences were then used as templates for sequence homology searches using BLAST. Searches were performed using the default parameters for BLASTx [26]. Alignments were generated using CLUSTAL [27]. Membrane-spanning helices were predicted using TMHMM [28]. Potential signal peptide cleavage sites were predicted

Table 2 Oligonucleotides used in this study

Oligonucleotide	Sequence (5′ → 3′)	Description
CiaB-DF	TAYACNCAYGGNGTNGC	Degenerate PCR
CiaB-DR	TCRTGNCCKATNGTNGA	
CiaB-F	GTGAACGATTTTAAAAGATTAAACG	Strain PCR
CiaB-R	CTATCTCTCGCTCGCTTGCC	
CiaB-RTF1	CTAGAACGCAAACCTACATCA	RT-PCR detection
CiaB-RTR1	CCGCTAAGCTTCATAAACGG	
M13 F	CGCCAGGGTTTTCCCAGTCACGA	DNA Sequencing
M13 R	TCACACAGGAAACAGCTATGAC	
CiaB-F5	CAAACTATAAAAATTTGGCGCTTC	
CiaB-R5	GAAAAGCTTTGCGAAAGTCG	
CiaB-Walk-5R	TACATCGCGTTTAGTTCGTCTTTGC	Genome Walker
CiaB-Walk-3F	CTATAGGAAACGAGCTTGACGAGAGC	
AP1	GAAAAGCTTTGCGAAAGTCG	
AP2	ACTATAGGGCACGCGTGGT	

N A, T, G, or C; *Y* C or T; *R* A or G; *K* G or T (IUPAC nucleotide ambiguity codes)

using SignalP [29]. PSORT-B was used to predict the cellular location of proteins [30].

Phylogenetic Analysis

A phylogenetic tree of *C. rectus* 314 CiaB was generated using Phylogeny-fr [31]. BLAST-EXPLORER [32] was used to retrieve and align sequences related to CiaB. After alignment, ambiguous regions were removed with Gblocks [33], and the resultant alignment was used to construct a tree using the maximum likelihood method [34, 35].

Conservation of CiaB Among *C. rectus* Strains

PCR was used to screen *C. rectus* DNA for the presence of *ciaB* genes. Primers specific to the 5′ and 3′ ends of *ciaB* from *C. rectus* 314 were used to PCR DNA from the *C. rectus* strains listed in Table 1.

Results

Identification of *C. rectus* 314 *ciaB*

Degenerate PCR was used to amplify a potential *ciaB* gene from DNA of *C. rectus* 314. PCR yielded an amplicon of the expected size (448 base pairs, Fig. 1). Amplicons of the correct size were also detected from *C. rectus* 33238 and *C. curvus* 33273. *C. curvus,* like *C. rectus*, is an oral bacteria associated with periodontitis [36]. BLASTx analysis of the amplicon from strain 314 revealed a gene fragment sharing sequence identity and similarity (53 and 70 %, respectively) to the [21] *ciaB* gene from *C. jejuni* (NCBI, AAD38497.1). The sequence of the 448 base pair amplicon was used as a template for PCR-based gene walking. PCR-based gene walking generated sequences upstream and downstream of the 448 base pair amplicon. Amplicons of 1,060 base pairs (downstream) and 892 base pairs (upstream) were cloned into pCR2.1-TOPO for sequencing (data not shown).

In Silico Analyses of *C. rectus ciaB*

Assembly of DNA sequences from PCR and gene walking revealed a *ciaB* ORF of 1,830 base pairs. The 1,830 base pair sequence is available at NCBI (#JN588592). The ORF included a start codon (GTG), stop codon (TAG), and ribosome-binding site 6 bases pairs upstream of the start codon (Shine-Delgaro sequence, AAAAGG). Translation of the ORF reveals a protein of 609 amino acids, with a molecular weight of 69.8 kDa. BLASTx analysis of the *C. rectus* 314 *ciaB*, named *Cr-ciaB*, revealed sequence identity and similarity to genes annotated as a hypothetical protein, a lipoprotein signal peptidase, and invasion antigen B (*ciaB*) proteins (Table 3). An alignment (Fig. 2) of Cr-CiaB showed homology with CiaB proteins, including the presence of a zinc-binding domain conserved among CiaB [22].

To further characterize Cr-CiaB, the protein was analyzed using tools designed to predict select cellular properties of genes. Specifically, *Cr-ciaB* was predicted not to contain membrane helices, did not contain a signal peptide, and is localized to the cytoplasm (Table 4). An identical analysis using related sequences (Table 3), including the lipoprotein signal peptidase from *C. rectus* 33238, a hypothetical protein from *C. curvus*, and CiaB proteins from related campylobacters; predicted a lack of membrane

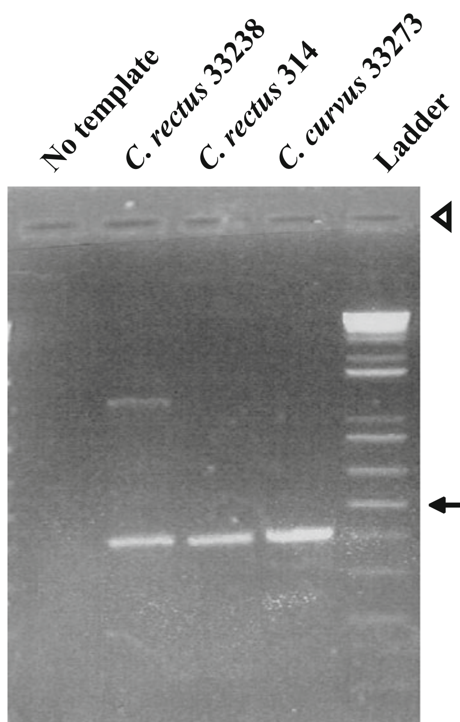

Fig. 1 Agarose gel of *C. rectus* or *Campylobacter curvus* DNA amplified by degenerate PCR (Table 1). An *arrow* marks 500 base pairs. *Ladder* 1 Kb plus DNA ladder (Invitrogen); and the *arrowhead* marks loading wells

Table 3 BLASTx analysis of *C. rectus* strain *ciaB*

Species[a]	Protein length (aa)	E-value	Annotation (GenBank #)	Identity (%)
C. rectus (strain 33238)	609	0.0	Lipoprotein signal peptidase (ZP03610758.1)	100
C. curvus	608	0.0	Hypothetical protein (YP001407631.1)	64
C. concisus	614	0.0	Invasion antigen B (EAT98433.1)[b]	60
C. fetus	606	0.0	Invasion antigen B (YP892436.1)	60
C. jejuni	610	0.0	Invasion antigen B (AAD38497.1)[c]	53
C. lari	611	0.0	Invasion antigen B (BAH23913.1)[d]	51

Representative BLASTx hits sharing identity with *C. rectus ciaB* (314)

[a] All species listed are campylobacters

[b] The gene is also annotated as a lipoprotein signal peptidase

[c] [15]

[d] [19]

Table 4 Predicted properties of *C. rectus* CiaB and related sequences

Species/gene[a]	Membrane helices[b]	Signal peptide probability[c]	Location (score)[d]
C. rectus 314 CiaB (JN588592)	No	0.0	Cytoplasm (8.96)
C. rectus 33238 signal peptidase (ZP03610758.1)	No	0.0	Cytoplasm (8.96)
C. curvus hypothetical protein (YP 001407631.1)	No	0.0	Cytoplasmic (8.96)
C. concisus CiaB (EAT98433.1)	No	0.0	Cytoplasmic (8.96)
C. fetus CiaB (YP 892436.1)	No	0.0	Cytoplasmic (8.96)
C. jejuni CiaB (AAD38497.1)	No	0.0	Cytoplasmic (8.96)
C. lari CiaB (BAH23913.1)	No	0.0	Cytoplasmic (8.93)

[a] Sequences as presented in Table 3

[b] [25]

[c] [26]

[d] [27]

Table 5 Properties of CiaB versus lipoprotein signal peptidases (LspA)

Species/gene[a]	Membrane helices[b]	Protein length (aa)	Cellular location (score)[c]
C. rectus 314 CiaB (JN588592)	No	609	Cytoplasm (8.96)
C. rectus 33238 signal peptidase (ZP03610758.1)[d]	No	609	Cytoplasm (8.96)
C. concisus CiaB[d] (EAT98433.1)	No	614	Cytoplasm (8.96)
C. jejuni CiaB[d] (AAD38497.1)	No	610	Cytoplasm (8.96)
Escherichia coli LspA (P00804)	Yes, 4	164	Membrane (9.82)
C. jejuni LspA (Q9PIE1)	Yes, 4	156	Membrane (10.0)
C. rectus 33238 signal peptidase II (ZP03609438.1)[e]	Yes, 4	188	Membrane (9.86)

[a] GenBank accession numbers are indicated in parentheses

[b] [25]

[c] [26]

[d] Identical to those genes in Table 3

[e] Signal peptidases are also referred to as signal peptidase II [37]

```
C.rectus 314    MNDFKRLNELVAVGKDELNAMYRQLDNP--SEVVLKMLKIAGFKGQKSEKIAVLRRIVDL 58
C.concisus      MNDFKRLNELTKEQKSKLNAIYKNLDN----ELINEAVKICGLTGTPSQKLALARRTVDL 56
C.fetus         MNDFAALNKLVKKQKNELNYLYKNMDN----EIVSKALNLCSLDSSSVNKIAVLRRIVDL 56
C.jejuni        MNNFKEIAKLVRKYKERNNALYEFLDKEDVGEYFRSLISLSELKQDKTTMLAILRRLIDL 60
                **:*  : :*.   *.. * :*. :*:    * . . :.:. :       :*: ** :**

C.rectus 314    KTEPLENELKKQGREEAAIGRIKDEAFELVREFYEARFERLLARIKEEKILDEFYSALLS 118
C.concisus      KVDPLQNELKKLNLGEDEQKRVLNLIYGYVRNLYENLHAQLIKKARDEQILDQFYQAFVE 116
C.fetus         KEEGIINELKNSGKNEEEIDIIKAKMYDFVSEFYINRHLNLINEVEEKGILEPFYLELIK 116
C.jejuni        KEENLVQEWKKNNFKEDKIIELKHKFYEEVRKFYEKEHQNLINEIKEKKLLNNFYQSLIQ 120
                * : : :* *: .  *     :    :  * ::*   . .*: . .:: :*: **  ::.

C.rectus 314    GMHGAGLAMNAWQSAWDRQILQTTNKEFEAKFASMAEAIKFIDENRLYQKDG-CEKGERS 177
C.concisus      AMHELGLSLNAWQISWQDKIIDTTNKEFEAKFKDLSEANEFITKNGLFQCDANGVRADRT 176
C.fetus         SVHRIGVSLSKMQKTWQKSIIETTNREFETKFESIAKANEFITKNKLYQINPDNSRCDRV 176
C.jejuni        GVHNIGLIMNIFEISWTKEIIEKNNKILSTQFPNLDDAMEFLRKNHLYQKTPEGEICERS 180
                .:*  *: :.  : :*  .*::..*: :.::* .: .* :*: :* *:*        :*

C.rectus 314    YGAVIKNGEKYAFAPYAAAFEKEVKRVADALDALIKNLKTLAANDEQRAYVTYFEKLKAA 237
C.concisus      YGAVCKEGEKFSFLPYALAFKDEVKELKKVFIKNLEILRNLAESDEQKSYVKYLEKLQNA 236
C.fetus         YGAVIKNGENYRLVPYAVSFKDEMKSVKDAFETSLANLEKLSKIKEDESYVIYLTKLKNA 236
C.jejuni        YGVLVRIGNLWKFVPYARFFENEILKLEFAFENMIDQLKIFASSEEEKAYIEYFEKLKLA 240
                **.: : *: : : ***  *:.*:  :  .:   :  *. ::  .*:.:*: *: **: *

C.rectus 314    FCERENDRAIPAWQDAERAWMDVKGPLQPGHPLEYYEDAYTHAVALEWDVRLAGTSDFDE 297
C.concisus      FCEEDNAKVINAWQEAEIAWMDVKGALQPGHPLEYYEDAYTHAVALEWDIRLVDSEGIDE 296
C.fetus         FLQTDNDLVIASWQDAERAWMDTKGKIQIGHPLEYYEDAYTHAVALEWDIRLVDDLSIDE 296
C.jejuni        FCEKDEDRVIKAWQEAEFAWMKVKSPLQVGHPLEYYEDNYTHAVALEWDIRIEDENDFDV 300
                * : ::  .* :**:** ***..*. :* ********* **********:*: .  .:*

C.rectus 314    AKFKKGVIRAYEQICEQCGIKDAALERQVTQNVARTQTYISVPMIYYGAELNGLFSAQVV 357
C.concisus      LKFKEKVAKTYKIVCEKIKFDNAETNKAVSENIARTQLYISVPMIYYGAELNGLFSAQVV 356
C.fetus         TKFKNSIKESFDEIYSKTGVKNQNMVNLVHSNIDKTQLYISGPMVYYAAEFNGLFSAQVV 356
C.jejuni        LKFGNEIKESFEHVYKNIGLEDCELEKEVLSNIEKTQLYICTPMIFYGAELKGLFSAQVV 360
                 ** : : .::. : .:  ..:    . * .*: :** **. **::*.**::********

C.rectus 314    PNDETVSKERGKKIFAFVEHVYESAKARPFMKLSGEIFSREFLDYGREILFKKPQIWKKT 417
C.concisus      PNDESVSAKCGKKIFAFVNHVYEGAKAKPFMKLGAEIFSKEFLDFGREILFLKPKIWKKV 416
C.fetus         PNDEIVSRECGKKIFAFVNFVYESTKAKPFMKLASEIFDKNFLDFGREILYKKPEIWKKV 416
C.jejuni        PNDEFVSSKAGKKIFAFINFVYENAKTKPFMKISSEVFDKEFLDFGRNILFYQEKIWKRV 420
                **** ** : *******::.***.:*::****:..*:*.::***:**:**: : :***:.

C.rectus 314    YEISTIGHEFGHILFVGTDTEKTMNADGEFKFIEEYKATTGGLVNFFIGGDASYDMSVFH 477
C.concisus      YEISTIGHEFGHILFIGLDTEMSMNKSGVFKFIEEYKATTGGLVNFFLHEEAEYKMAVFH 476
C.fetus         YEISTIGHEFGHILFIDSDTESAMNKSGEFKFIEEYKATTGGLINFFLHEDDTYKMAVFH 476
C.jejuni        YEVSTIGHEFGHIFFIANDTEKTMNQSGFFKNIEEYKATTGGLINFFYHEQDDLIMPVFH 480
                **:**********:*:  *** :** .* ** ***********:***   :    *.***

C.rectus 314    ELIARAVGLIAWREVDEVRAYYCEGLIHLSLLFDSGALSFEDGALIVKFDREHYAKFKEI 537
C.concisus      ELIARAVGLIAWRKVDEVRAYYCEGLIHLSLLFRAGVLKFDG-KLSVDMSEQAYAKFKEI 535
C.fetus         ELIKISVGLIAWQKVTETRAYYCEGLIHLSLLFRSGVLKFDGKKLQIDFTQNGYQNFKNE 536
C.jejuni        ELIKRAIGLISWQRVDEVRPYYTEGLIHLSLLFESEVLIFENNNLKINFDLGHYEKFKEL 540
                ***  ::***:*:.* *.*.** ********** : .* *:.  * :.:    * :**:

C.rectus 314    CLANYKNLALHYARRAPAGEFLAKFCDKEGKSYLPKHAQARAFVEHYYARYKAIGNELDE 597
C.concisus      CLQNYFDLAQTYAKKDDASTFLEKFCQKDELSYLPKDEECKKFVEHFYARYEAIGNDVDD 595
C.fetus         VMKNYINLATHYSNKLNAGEFLAKFAKFEGEIYLPKNVETLEFVKFYYQKYEKMANEIDD 596
C.jejuni        TLKNYHELAKHYALRLDAKEFLSRFCEIEDNIFLPIMPKCKEFVKFYYDLYEKIGNEIDN 600
                 : ** :**  *: :  *  ** :*.. :   :**   :   **:.:*  *: :.*::*:

C.rectus 314    SGEWEKWQASER------- 609
C.concisus      SGEWQRWQKLAQESESGRA 614
C.fetus         SGEWQKWQQA--------- 606
C.jejuni        SGEFERYKKK--------- 610
                ***:::::
```

Fig. 2 Alignment of *Campylobacter rectus* 314 CiaB (GenBank # JN588592) with putative homologues from: *Campylobacter concisus* (EAT98433.1, Table 3); *Campylobacter fetus* (YP 892436.1, Table 3); and *Campylobacter jejuni* (AAD38497.1, Table 3). An *asterisk* indicates a conserved residue, moderate conservation is marked by a *colon*, and a *period* indicates weak conservation. *Grey* denotes a zinc-binding domain conserved among CiaB [19]

helices, a lack of signal peptides, and a cytoplasmic localization (Table 4). For predictions using PSORT-B, a score of greater than 7.5 is considered significant.

Analysis of *Cr-ciaB* showed sequence identity (Table 3, ZP03610758.1) with a gene from *C. rectus* 33238 annotated as a lipoprotein signal peptidase (Cr-LspA). However,

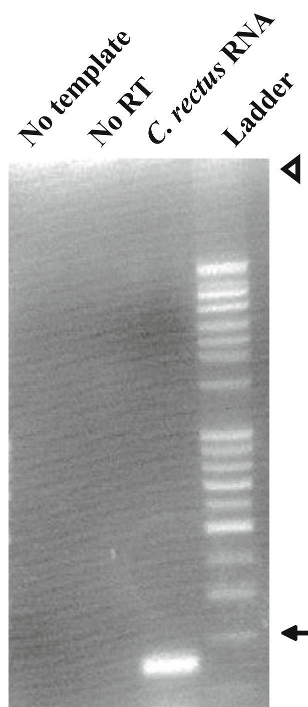

Fig. 3 Agarose gel of *C. rectus strain* 314 RNA amplified with RT-PCR. *No template* no RNA template control, *No RT* no reverse-transcriptase control, *Ladder* 1 Kb plus DNA ladder. An *arrow* marks 100 base pairs; and the *arrowhead marks* loading wells

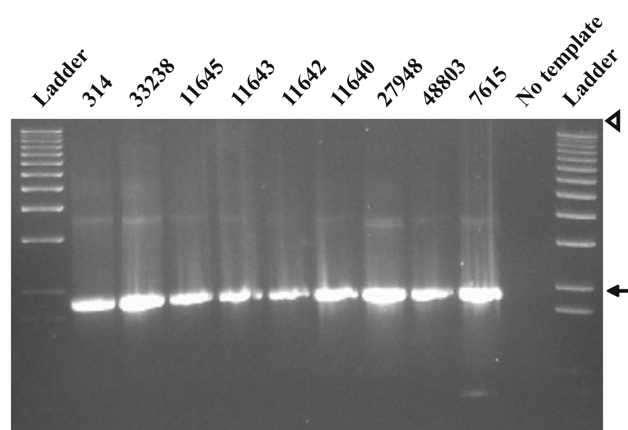

Fig. 4 Agarose gel of *C. rectus* DNA amplified using primers designed to amplify the complete *ciaB* gene (Table 2). Each *number* refers to a different strain of *C. rectus* (Table 1). An *arrow* marks 2,000 base pairs. Ladder = 1 Kb Plus DNA ladder; and the *arrowhead* denotes loading wells

in comparing the predicted properties of Cr-CiaB and Cr-LspA to the characteristics of bacterial LspA from *Escherichia coli* and *C. jejuni* [37], significant differences were observed. In contrast to Cr-CiaB and Cr-LspA, the bacterial LspA are predicted to contain membrane helices and are likely to be located within membranes (Table 5). For *E. coli* LspA, the membrane helices and cellular location have been demonstrated [38]. In addition, bacterial LspA are less than 200 amino acids long. In contrast, CiaB proteins are greater than 600 amino acids (Table 5).

Expression of *Cr-ciaB* and Conservation Among *C. rectus* Strains

Expression of *Cr-ciaB* was shown by RT-PCR (Fig. 3). In addition, PCR suggests that CiaB is conserved among *C. rectus* strains (Fig. 4).

Phylogeny of Cr-CiaB

A maximum likelihood tree of Cr-CiaB and related sequences is depicted in Fig. 5. As shown, CiaB proteins from related bacteria form a major branch of the tree that is significantly supported (100 %). Within the main branch, a cluster of related CiaB proteins from campylobacters was noted. The CiaB proteins among the campylobacters are sorted according to species, including a group of CiaB from the oral campylobacters. Significantly, Cr-CiaB and the gene from *C. rectus* 33238 annotated as a lipoprotein signal peptidase clearly places both genes among CiaB proteins.

Discussion

In this study a *ciaB* gene from *C. rectus* 314, a poorly understood periodontal pathogen, was identified and characterized. Initial analysis of *C. rectus* 314 *ciaB* (*Cr-ciaB*) was surprising as BLASTx revealed 100 % sequence identity to a *C. rectus* 33238 gene annotated as a lipoprotein signal peptidase. However, further characterization of the 33238 gene; along with parallel studies of *C. curvus* YP001407631.1 and *Campylobacter concisus* EAT98433.1, suggests that all three genes represent CiaB proteins, not lipoprotein signal peptidases. The possibility that the genes from *C. rectus* 33238, *C. curvus,* and *C. concisus* were annotated incorrectly is plausible. Indeed, between 30 and 40 % of annotated microbial genes are assigned incorrect functions using automated software [39]. Of course, future biochemical and genetic studies are needed to fully define the function of *C. rectus* 314 *ciaB.*

CiaB genes have been identified from several species of campylobacters that do not typically reside in the oral cavity of humans including *C. jejuni*, *C. lari*, *C. coli*, *C. fetus,* and *C. upsaliensis* [21, 22]. However, during the course of characterizing the *ciaB* from *C. rectus* 314, potential *ciaB* genes were identified among campylobacters that do reside within the oral cavity of humans (Table 3) including *C. rectus* 33238 (ZP03610758.1, annotated at NCBI as a lipoprotein signal peptidase), *C. curvus* (YP001407631.1, annotated as a hypothetical protein), and *C. concisus* (EAT98433.1,

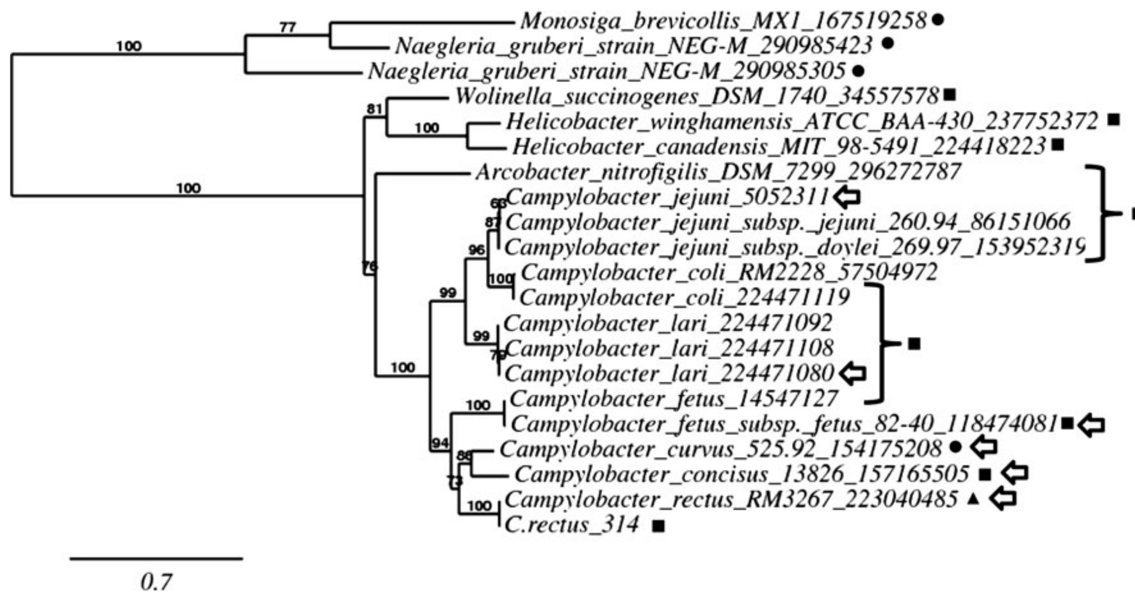

Fig. 5 Phylogram of *C. rectus* strain 314 CiaB and related genes. *Values* at nodes represent branch support [35]. The NCBI gene identifier is indicated for each species. Proteins marked by a *square* are annotated as CiaB. Proteins marked with a *circle* are annotated as hypothetical proteins. The protein marked by a *triangle* is annotated as a lipoprotein signal peptidase. Proteins marked with an *arrow* are those presented in Table 3. The *scale bar* represents evolutionary distance (0.7)

annotated as both lipoprotein signal peptidase and an invasion antigen B). The conservation of CiaB proteins among oral and non-oral species suggests that CiaB may be important to the lifestyle of campylobacters, independent of niche preference. In addition, this study represents the first effort to characterize a *ciaB* gene from any of the oral campylobacters.

Among the best studied virulence factors of campylobacters is the invasion antigen gene B (*ciaB*) of *C. jejuni*, which has been shown to play a key role in host cell invasion [18–20]. In *C. jejuni*, CiaB has been shown to be a secreted protein, although it lacks a predictable signal peptide [19]. Interestingly, *C. rectus* 314 CiaB also appears to lack a signal peptide (Table 3). As previously mentioned, *C. rectus* 314, like *C. jejuni*, has been shown to invade eukaryotic cells, including placental trophoblasts [23]. Invasion of placental trophoblasts is important, as previous studies have suggested that *C. rectus* plays a significant role in the preterm labors of mothers with periodontitis [10, 11] and has the ability to translocate to the placenta, from a distant site of infection, in mouse models of periodontitis.

Conclusions

The suite of genes that play a role in the pathogenesis of *C. rectus*, including those that contribute to the process of cell invasion, are unknown. However, the discovery of *C. rectus* 314 CiaB in this study, taken together with the observed cell invasion by *C. rectus*, suggests that CiaB may play a role in this phenotype [23]. Future studies aim to verify the function of CiaB biochemically. In addition, the identification of *C. rectus* CiaB raises the possibility of developing novel periodontal therapeutics that disrupt CiaB functionality.

Acknowledgments Michael J. LaGier was supported in part by Florida Gulf Coast University (Internal Grant #09101). This study was also supported in part by the NSF-POWRE MCB-9973861 to Deborah S. Threadgill.

References

1. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366:1809–1820
2. Teng YT et al (2002) Periodontal health and systemic disorders. J Can Dent Assoc 68:188–192
3. Colombo AV et al (2006) Identification of oral bacteria associated with crevicular epithelial cells from chronic periodontitis lesions. J Med Microbiol 55:609–615
4. Dzink JL et al (1985) Gram negative species associated with active destructive periodontal lesions. J Clin Periodontol 5:648–659
5. Lai CH et al (1992) *Wolinella recta* in adult gingivitis and periodontitis. J Periodontal Res 27:8–14
6. Bobetsis YA, Barros SP, Offenbacher S (2006) Exploring the relationship between periodontal disease and pregnancy complications. J Am Dent Assoc 137:7S–13S
7. Offenbacher S et al (1996) Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 67:1103–1113
8. Madianos PN et al (2001) Maternal periodontitis and prematurity Part II: maternal infection and fetal exposure. Ann Periodontol 6:175–182

9. Borinski R, Holt SC (1990) Surface characteristics of *Wolinella recta* ATCC 33238 and human clinical isolates: correlation of structure with function. Infect Immun 58:2770–2776
10. Offenbacher S et al (2005) Effects of maternal *Campylobacter rectus* infection on murine placenta fetal and neonatal survival and brain development. J Periodontol 76:2133–2143
11. Yeo A et al (2005) *Campylobacter rectus* mediates growth restriction in pregnant mice. J Periodontol 76:551–557
12. Hofreuter D et al (2006) Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect Immun 74:4694–4707
13. Pearson BM et al (2007) The complete genome sequence of *Campylobacter jejuni* strain 81116 (NCTC11828). J Bacteriol 189:8402–8403
14. Braun M et al (1999) Cloning and characterization of two bi-structural S-layer-RTX proteins from *Campylobacter rectus*. J Bacteriol 181:2501–2506
15. LaGier MJ, Threadgill DS (2008) Identification of novel genes in the oral pathogen *Campylobacter rectus*. Oral Microbiol Immunol 2008(23):406–412
16. Wang B, Kraig E, Kolodrubetz D (2000) Use of defined mutants to assess the role of the *Campylobacter rectus* S-layer in bacterium-epithelial cell interactions. Infect Immun 68:1465–1473
17. Young KT, Davis LM, Dirita VJ (2007) *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol 5: 665–679
18. Konkel ME et al (1999) Bacterial secreted proteins are required for the internaliztion of *Campylobacter jejuni* into cultured mammalian cells. Mol Microbiol 32:691–701
19. Konkel ME et al (2004) Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. J Bacteriol 186:3296–3303
20. Malik-Kale P, Parker CT, Konkel ME (2008) Culture of *Campylobacter jejuni* with sodium deoxycholate induces virulence gene expression. J Bacteriol 190:2286–2297
21. Fouts DE et al (2005) Major structural differences and novel potential virulence mechanisms from the genomes of multiple Campylobacter species. PLoS Biol 3:e15
22. Onozato J et al (2009) Cloning sequencing and expression of full-length Campylobacter invasion antigen B gene operon from *Campylobacter lari*. J Basic Microbiol 49:342–349
23. Arce RM et al (2010) Characterization of the invasive and inflammatory traits of oral *Campylobacter rectus* in a murine model of fetoplacental growth restriction and in trophoblast cultures. J Reprod Immunol 84:145–153
24. Ausubel F et al (1990) Current protocols in molecular biology. Wiley, New York
25. Wren BW et al (1992) Degenerate PCR primers for the amplification of fragments from genes encoding response regulators from a range of pathogenic bacteria. FEMS Microbiol Lett 78:287–291
26. Altschul S et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410
27. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
28. Krogh A et al (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580
29. Emanuelsson O et al (2007) Locating proteins in the cell using TargetP SignalP and related tools. Nat Protoc 2:953–971
30. Rey S et al (2005) PSORTdb: a protein subcellular localization database for bacteria. Nucleic Acids Res 33:D164–D168
31. Dereeper A et al (2008) Phylogenyfr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:W465–W469
32. Dereeper A et al (2008) BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol 10:8
33. Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 2007(56):564–577
34. Guindon S et al (2005) PHYML online–a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res 33:W557–W559
35. Chevenet F et al (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinform 7:439
36. Mucach P, Tanner AC (2000) Campylobacter species in health gingivitis and periodontitis. J Dent Res 79:785–792
37. Paetzel M et al (2002) Signal peptidases. Chem Rev 102:4549–4580
38. Yu F et al (1984) Nucleotide sequence of the lspA gene the structural gene for lipoprotein signal peptidase of *Escherichia coli*. FEBS Lett 173:264–268
39. Poptsova MS, Gogarten JP (2010) Using comparative genome analysis to identify problems in annotated microbial genomes. Microbiology 156:1909–1917