Zonula occludens toxins and their prophages in Campylobacter species
Fang Liu, Hoyul Lee, Ruiting Lan and Li Zhang*

Abstract

Background: We previously showed that zonula occludens toxin (Zot) encoded by Campylobacter concisus zot^808T gene has the potential to initiate inflammatory bowel disease. This Zot protein caused prolonged intestinal epithelial barrier damage, induced intestinal epithelial and macrophage production of tumor necrosis factor-α and enhanced the responses of macrophages to other microbes. In order to understand the potential virulence of Zot proteins in other Campylobacter species, in this study we examined their presence, similarities, motifs and prophages.

Methods: The presence of Zot proteins in Campylobacter species was examined by searching for the Zot family domain in multiple protein databases. Walker A and Walker B motifs in Zot proteins were identified using protein sequence alignment. A phylogenetic tree based on Campylobacter zot genes was constructed using maximum-likelihood method. Campylobacter Zot proteins were compared using protein sequence alignment. The zot-containing prophages in Campylobacter species were identified and compared with known prophage proteins and other viral proteins using protein sequence alignment and protein BLAST.

Results: Twelve Zot proteins were found in nine Campylobacter species/subspecies. Among these Campylobacter species, three species had two Zot proteins and the remaining six species/subspecies had one Zot protein. Walker A and Walker B motifs and a transmembrane domain were found in all identified Campylobacter Zot proteins. The twelve Campylobacter zot genes from the nine Campylobacter species/subspecies formed two clusters. The Zot\textsubscript{CampyType_1} proteins encoded by Cluster 1 Campylobacter zot genes showed high similarities to each other. However, Zot\textsubscript{CampyType_2} proteins encoded by Cluster 2 Campylobacter zot genes were more diverse. Furthermore, the zot-containing Campylobacter prophages were identified.

Conclusion: This study reports the identification of two types of Campylobacter Zot proteins. The high similarities of Zot\textsubscript{CampyType_1} proteins suggest that they are likely to have similar virulence. Zot\textsubscript{CampyType_2} proteins are less similar to each other and their virulent properties, if any, remain to be examined individually.

Keywords: Campylobacter concisus, Zonula occludens toxin (Zot), Campylobacter, Prophage

Background

Campylobacter concisus is a Gram-negative spiral shaped motile bacterium [1]. Their growth under both anaerobic and microaerobic conditions is largely determined by the presence of H\textsubscript{2} [2]. This bacterium usually colonizes the human oral cavity [3, 4]. However, it may also colonize the intestinal tract of some individuals and its prevalence in intestinal biopsies has been associated with human inflammatory bowel disease (IBD) [5–9]. Translocation of C. concisus from the oral cavity to intestinal tract has been suggested to be a cause of a subgroup of IBD [10]. Campylobacter concisus has also been suggested to be involved in diarrheal disease due to the frequent isolation of this bacterium from diarrheal stool samples [8, 11–13].

Campylobacter concisus zonula occludens toxin (Zot) is encoded by the zot gene located in CON\_phi2 prophage [14, 15]. The zot genes in different C. concisus strains have polymorphisms [14]. In a recent study, we examined the effects of C. concisus Zot encoded by zot^808T gene on human intestinal epithelial cells and macrophages using cell line
models. In that study, we found that *C. concisus* Zot caused prolonged intestinal epithelial barrier damage, induced intestinal epithelial and macrophage production of proinflammatory cytokines such as tumor necrosis factor-α, and enhanced the responses of macrophages to *Escherichia coli* [16]. These data suggest that *C. concisus* Zot may play a role in initiating chronic intestinal inflammatory conditions such as IBD through damaging the intestinal barrier and enhancing the immune responses to luminal microbes.

In addition to *C. concisus*, four additional *Campylobacter* species including *Campylobacter ureolyticus*, *Campylobacter coragaei*, *Campylobacter gracilis* and *Campylobacter iguaniorum* were recently reported to possess the zot gene [17–21]. The similarities of Zot proteins in different *Campylobacter* species have not been examined. In this study, we examined Zot proteins and their genes in *Campylobacter* species, which revealed two clusters of *Campylobacter zot* genes and two types of *Campylobacter* Zot proteins. Furthermore, we identified the prophages that contain zot genes in *Campylobacter* species.

**Methods**

**Examination of the presence of Zot proteins in *Campylobacter* species**

The presence of Zot proteins in *Campylobacter* species was examined by searching for proteins in *Campylobacter* species that have the Zot family domain in multiple protein databases including NCBI protein database, InterPro and Pfam [22–24]. The Zot proteins in these databases were annotated based on the presence of Zot family domain.

**Examination of the presence of Walker A and Walker B in *Campylobacter* Zot proteins**

It is shown in the InterPro database that the Zot family proteins (InterPro entry identity: IPR008900) belong to p-loop containing nucleoside triphosphate hydrolase (p-loop NTPase) superfamily. The proteins of p-loop NTPase superfamily have Walker A and Walker B motifs [25]. In this study, we examined the presence of Walker A and Walker B motifs in *Campylobacter* Zot proteins by protein alignment using Clustal Omega software [26]. The Walker A motif has a sequence of GxxxxGK[S/T], where x is any residue and the Walker B motif has a sequence of hhhh[D/E], where h is a hydrophobic residue [25].

**Generation of a phylogenetic tree based on zot genes in *Campylobacter* species**

To examine the genetic relationship between the zot genes in different *Campylobacter* species, the nucleotide sequences of the zot genes in *Campylobacter* species identified above were obtained from NCBI genome database. These sequences were used to generate a phylogenetic tree using the maximum likelihood method implemented in molecular evolutionary genetics analysis software version 6.0 [27]. In order to differentiate the zot genes in different *Campylobacter* species, the zot genes found in different *Campylobacter* species were indicated by the last four digits of their locus tag numbers.

**Comparison of zot-containing prophages in *Campylobacter* species**

To examine whether the zot genes in different *Campylobacter* species are carried by prophages similar to that in *C. concisus*, the zot-containing prophages in these *Campylobacter* species were identified by examination of the genes adjacent to their zot genes. The prophages were defined based on the presence of integrase, hypothetical proteins and attachment sites [28]. The attachment sites were identified by the presence of repetitive sequences located at both ends of the prophages [28].

The proteins in the identified *Campylobacter* prophages in this study were compared with the corresponding proteins in *C. concisus* prophages CON_phi2 and CON_phi3. The comparison was performed using Clustal Omega [26]. Proteins sharing more than 40 % sequence identity were considered to have high similarities and were recorded [29].

**Comparison of the proteins of zot-containing prophages in *Campylobacter* species with other viral proteins**

To examine whether the zot containing prophages identified in *Campylobacter* species are similar to previously reported prophages, proteins of *Campylobacter* prophages were compared with viral proteins in the NCBI non-redundant protein sequence database (taxonomy identity for viruses: 10,239) using protein BLAST with default settings [22]. The identified viral proteins with E-values lower than 10 were noted. For prophage proteins which shared sequence similarities with multiple viral proteins, the viral proteins with the lowest E-values were noted. The full length sequences of the identified viral proteins were then aligned with the *Campylobacter* prophage proteins using Clustal Omega [26]. The protein identities were calculated by dividing the number of identical amino acids by the total number of amino acids in proteins from *Campylobacter* prophages. The *Campylobacter* prophage proteins were also compared with viral proteins in the virus pathogen database and analysis resource (ViPR) using BLAST with a cut-off E-value of 10. In addition to *C. concisus*, the zot gene was also found in other bacterial species such as *Vibrio cholerae* and *Neisseria meningitis* [30, 31]. The
identities between *Campylobacter* Zot, *V. cholerae* Zot and *N. meningitidis* Zot proteins were also compared in this study.

**Prediction of secreted proteins and transmembrane proteins in Campylobacter prophages**

Secreted proteins in *zot*-containing prophages in different *Campylobacter* species were predicted using SignalP 4.1 and SecretomeP 2.0. The software SignalP 4.1 predicts secreted proteins based on the presence of the signal peptide at the N-terminus [32]. The software SecretomeP 2.0 predicts non-classical (not signal peptide triggered) protein secretion based on analysis of post-translational and localizational aspects of the proteins [33]. Transmembrane proteins were predicted using the software Phobius [34].

**Results**

**The Zot proteins in different Campylobacter species and their Walker A and Walker B motifs**

Twelve Zot proteins were found in nine *Campylobacter* species/subspecies. Three *Campylobacter* species including *C. concisus*, *C. ureolyticus* and *C. corcagiensis* had two Zot proteins and the remaining six *Campylobacter* species/subspecies including *C. gracilis*, *Campylobacter jejuni* subsp. *doylei*, *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *C. hyointestinalis* subsp. *lawsonii* and *C. iguaniorum* had one Zot protein (Table 1).

The Zot family domains were localized at the N-terminal side of the identified *Campylobacter* Zot proteins, prior to the transmembrane domains. The Zot family proteins had p-loop NTPase domains and the entry identity for p-loop NTPase domain was IPR027417 in InterPro database. We identified the Walker A and Walker B motifs in *Campylobacter* Zot proteins, which were at the N-terminal side of the Zot proteins (Fig. 1).

The phylogenetic tree formed based on *zot* genes in *Campylobacter* species

The *Campylobacter* *zot* genes formed two clusters. Cluster 1 contained three *zot* genes, including *C. concisus zot2276*, *C. ureolyticus zot3935* and *C. corcagiensis zot6485*. Cluster 2 contained nine *Campylobacter* *zot* genes (Fig. 2).

**Comparison of ZotCampyType_1 and ZotCampyType_2 Proteins**

The Zot proteins encoded by Cluster 1 and Cluster 2 *Campylobacter* *zot* genes were referred to as ZotCampyType_1 and ZotCampyType_2 proteins respectively. The three ZotCampyType_1 Proteins had high similarities; they shared 171 identical amino acids and 77 conservative mutations (Fig. 3). The ZotCampyType_2 proteins were less similar to each other as compared to ZotCampyType_1 proteins; they had 71 identical amino acids and 65 conservative mutations (Fig. 4).

**The *zot*-containing prophages in different Campylobacter species and their similarities to *C. concisus* prophages CON_phi2 or CON_phi3**

We identified the *zot*-containing prophages in different *Campylobacter* species. Each of these prophages began with an integrase and had a number of hypothetical proteins (Fig. 5; Additional file 1). The attachment sites were found (Table 2). These prophages were inserted within either tRNA-Met or tRNA-Ser genes, except for URE_phiZB, which was inserted into tRNA-Leu gene (Table 2). For the prophage in *C. iguaniorum*, two tRNA genes were found after the integrase, suggesting that multiple insertions have occurred.

### Table 1 Zot proteins in *Campylobacter* species/subspecies

| *Campylobacter* species/subspecies | Strain | Number of *Zot* proteins | Locus tag | Source of isolation | Reference |
|-----------------------------------|--------|---------------------------|-----------|---------------------|-----------|
| *C. concisus*                     | 13826  | 2                         | CCC13826_2276, CCC13826_0191 | Human faeces | Gb0000058a |
| *C. ureolyticus*                  | DSM 20703 | 2                     | CS12_RS0103935, CS12_RS01000745 | Human amniotic fluid | [35] |
| *C. corcagiensis*                 | CIT045 | 2                         | BG71_RS0106485, BG71_RS0104620 | Lion-tailed macaques faeces | [36] |
| *C. gracilis*                     | RM3268 | 1                         | CAMGR0001_2456 | Human oral cavity | Gb0003988a |
| *C. jejuni* subsp. *doylei*       | 269.97 | 1                         | JD26997_0348 | Human blood | Gb0000076a |
| *C. jejuni* subsp. *jejuni*       | 60004  | 1                         | CJ111_RS08060 | Chicken | SAMN02429007 |
| *C. hyointestinalis* subsp. *hyointestinalis* | DSM 19053 | 1                     | CR67_RS1870 | Porcine intestine | SAMN01176354 |
| *C. hyointestinalis* subsp. *lawsonii* | CCUG 27631 | 1                     | CHL_RS06765 | Porcine intestine | [37] |
| *C. iguaniorum*                   | RM11343 | 1                         | CIG11343_RS03950 | Alpaca faeces | [21] |

* Genome online database project ID. **NCBI Biosample ID
Prophages containing Zot\textsubscript{CampyType\_1} proteins had high similarities to CON\_phi2. Eight proteins in URE\_phiZA and nine proteins in COR\_phiZA were found to have more than 40\% identities (41-73\%) with proteins in CON\_phi2 (Fig. 5a; Additional file 1). Proteins in prophages containing Zot\textsubscript{CampyType\_2} proteins were more diverse. However,
**Fig. 2** The phylogenetic tree generated based on zot genes in different *Campylobacter* species. Maximum likelihood method was used to generate the phylogenetic tree. Bootstrap values were generated from 1000 replicates. Cluster 1 zot genes are shown in bold. The zot gene from *Neisseria meningitidis* (strain 69166) was used as the outgroup.

**Fig. 3** Comparison of ZotCampyType_1 proteins. The protein similarities were compared using Clustal Omega. Asterisk indicates identical amino acids (shaded in red). Colon indicates conservative mutations (shaded in blue). Dot indicates semi-conservative mutations. Transmembrane domains are underlined.
Fig. 4 Comparison of ZotCampyType_2 proteins. The protein similarities were compared using Clustal Omega. Asterisk indicates identical amino acids (shaded in red). Colon indicates conservative mutations (shaded in blue). Dot indicates semi-conservative mutations. Transmembrane domains are underlined.
three to five proteins in these prophages had more than 40% identities with that in CON_phi3 (40–72%) (Fig. 5b; Additional file 1).

The similarities between proteins in Campylobacter prophages and viral proteins

The zot-containing Campylobacter were compared with known viral proteins in NCBI non-redundant protein sequence database. The proteins within each individual prophage showed low similarities to multiple phage proteins, except for CCC13826_0188 in CON_phi3 that shared 43% identity with a phage transferase from an uncultured phage (Additional file 2). The Zot proteins in Campylobacter prophages had low similarities with the Zot proteins in V. cholerae and N. meningitidis (15–21%) (Additional files 3, 4). None of the Campylobacter prophage proteins shared significant similarities with viral proteins in ViPR database. These data suggest that the zot-containing prophages in Campylobacter species are new prophages that have not been previously characterized.

Secreted and transmembrane proteins in Campylobacter prophages

Proteins secreted via both classical secretory pathway (with signal peptides) and non-classical secretory pathway (without signal peptides), as well as transmembrane proteins were found in all Campylobacter prophages (Fig. 5).

Discussion

In addition to C. concisus, a number of other Campylobacter species were recently reported to have the zot genes [17–21]. In this study, we found that Campylobacter zot genes formed two clusters (Fig. 2). Most of the Campylobacter zot genes were in Cluster 2, and Cluster 1 contained only three zot genes. The three Campylobacter species that had Cluster 1 zot genes also contained Cluster 2 zot genes. The remaining six Campylobacter species/ subspecies contained Cluster 2 zot genes only. These data show that Cluster 2 zot gene is more prevalent in Campylobacter species as compared to Cluster 1 zot genes.

Zot_CampyType_1 proteins, which were encoded by Cluster 1 zot genes, were highly similar to each other. However they were less similar to Zot_CampyType_2 proteins that were encoded by Cluster 2 Campylobacter zot genes. The zot gene in CON_phi2 prophage in C. concisus belongs to Zot_CampyType_1. Using cell culture models, we previously showed that Zot_CampyType_1 in C. concisus encoded by zot^808T polymorphism damaged intestinal epithelial barrier by induction of epithelial apoptosis and induced production of proinflammatory cytokines such as TNF-α in HT-29 cells and THP-1 macrophage-like cells, supporting its role as a potential virulence factor [16]. The high similarities between Zot_CampyType_1 proteins in the three different Campylobacter species suggest that they may have similar effects on human cells. Great variations in protein sequences between Zot_CampyType_1 and Zot_CampyType_2 proteins as well as within Zot_CampyType_2 proteins were observed in this study. Given this, the effects of Zot_CampyType_2 proteins on human cells, if any, require to be examined individually.

A transmembrane domain was found in all Zot proteins, showing that Zot proteins are transmembrane proteins. Furthermore, all Zot proteins contained Walker A and Walker B motifs, which are conserved motifs of p-loop NTPase superfamily [25]. P-loop NTPase bind to NTP typically ATP or GTP through the Walker A and B motifs, which are involved in diverse cellular functions [25]. Future studies should be conducted to examine whether Campylobacter Zot proteins have NTPase activities.

In this study, we identified a number of zot-containing prophages in other Campylobacter species in addition to previous reported prophages in C. concisus (Fig. 5). These prophages have an integrase, a number of hypothetical proteins and attachment sites (Fig. 5; Table 2; Additional file 1), which satisfy the previously defined criteria for prophages [28]. The proteins in individual Campylobacter prophages identified in this study have low similarities with multiple viral proteins, suggesting that they are new prophages that have not being characterized previously (Additional file 2).

Campylobacter Zot proteins had very low similarities to V. cholerae Zot and N. meningitidis Zot proteins (Additional files 3, 4). These data showed that despite having a common name, the amino acid sequences of Zot proteins in different bacterial species vary greatly. Thus, they may not necessarily exhibit the same effects on human cells.
Table 2 The attachment sites of zot-containing Campylobacter prophages

| Prophage          | Starta | Enda | Attachment gene sequence b | tRNA (locus_tag)                      |
|-------------------|--------|------|-----------------------------|--------------------------------------|
| CON_phi1, CON_phi2 and CON_phi3 (C. concisus 13826) | 1582286 | 1582311 | TCCAAATCCCTCCTGGGCCAACCA    | tRNA-Ser (CCC13826_RS07905)          |
|                   | 1587508 | 1587533 | TCCAAATCCCTCCTGGGCCAACCA    |                                      |
|                   | 1597113 | 1597138 | TCCAAATCCCTCCTGGGCCAACCA    |                                      |
|                   | 1606718 | 1606743 | TCCAAATCCCTCCTGGGCCAACCA    |                                      |
|                   | 1616160 | 1616185 | TCCAAATCCCTCCTGGGCCAACCA    |                                      |
| CON_phi4 (C. concisus 13826) | 946941 | 946993 | CTCAATAACCGGAAGTGGGGTTTCAA   | tRNA-Met (CCC13826_RS04780)          |
|                   | 937290  | 937342 | CTCAATAACCGGAAGTGGGGTTTCAA   |                                      |
| URE_phiZA (C. ureolyticus DSM 20703) | 68067 | 68122 | ATACGGGAGTGTGGGTTAAGTCTCTCT   | tRNA-Met (CS12_RS013965)             |
|                   | 57811  | 57866 | ATACGGGAGTGTGGGTTAAGTCTCTCT   |                                      |
| URE_phiZB (C. ureolyticus DSM 20703) | 139564 | 139610 | GTTCAAGTCTGCGTATGACACCATTA   | tRNA-Leu (CS12_RS010076)             |
|                   | 130103 | 130149 | GTTCAAGTCTGCGTATGACACCATTA   |                                      |
| COR_phiZA (C. corcagiensis CIT045) | 254051 | 254088 | CGAAGTCACGGGTTCAAGTCCCTTCT   | tRNA-Met (SA94_RS06290)              |
|                   | 265302 | 265339 | CGAAGTCACGGGTTCAAGTCCCTTCT   |                                      |
| COR_phiZB (C. corcagiensis CIT045) | 257240 | 257264 | GTTCAATCCCTCTCTGGGCCAACCA    | tRNA-Ser (SA94_RS04510)              |
|                   | 247319 | 247343 | GTTCAATCCCTCTCTGGGCCAACCA    |                                      |
| GRACI_phiZ (C. gracilis RM5268) | 112826 | 112871 | CTCATAACGGGAAGTGGGGTTTCAA   | tRNA-Met (CAMGR0001_2931)            |
|                   | 102518 | 102563 | CTCATAACGGGAAGTGGGGTTTCAA   |                                      |
| DOYLEI_phiZ (C. jejuni subsp. doylei 269.97) | 303215 | 303241 | AGGGTCTAATACTCTCTGCGGCCA    | tRNA-Ser (JJD26997_RS01570)          |
|                   | 313165 | 313191 | AGGGTCTAATACTCTCTGCGGCCA    |                                      |
| HYO_phiZ (C. hyointestinalis subsp. hyointestinalis DSM 19053) | 360409 | 360446 | CTCATAACGGGAAGTGGGGTTTCAA   | tRNA-Met (CR67_RS01810)              |
|                   | 369716 | 369753 | CTCATAACGGGAAGTGGGGTTTCAA   |                                      |
| HYO_phiZ (C. hyointestinalis subsp. lawsonii CCUG 27631) | 1283134 | 1283238 | CTCATAACGGGAAGTGGGGTTTCAA   | tRNA-Met (CHL_RS06785)               |
|                   | 1273720 | 1273824 | CTCATAACGGGAAGTGGGGTTTCAA   |                                      |
|                   |        |        | ATCATTCTTCTGCGGCCAACAA      |                                      |

a The start and end positions for the attachment sites refer to the nucleotide position within the contig containing the prophage genomes, except for C. concisus strains 13826, C. jejuni subsp. doylei 269.97 and C. hyointestinalis subsp. lawsonii CCUG 27631 which refer to the nucleotide position in the full genome.
b Attachment sites overlapped with 3’ end of tRNA, the overlapped sequences were italic.
c Multiple insertion sites for CON_phi prophages in C. concisus 13826 in which only the first attachment site (for CON_phi1) overlapped with tRNA. In NCBI database, the contig encoding JEUNI_phiZ did not cover the full prophage genome; therefore it was unable to locate the attachment sites. No attachment site was identified in IGUA_phiZ.

Conclusions
This study reports the identification of two types of Campylobacter Zot proteins. The high similarities of ZotCampyType_1 proteins suggest that they are likely to have similar virulence. ZotCampyType_2 proteins were less similar to each other and their virulent properties, if any, remain to be examined individually. This study provides useful information for further examination of Campylobacter Zot proteins as potential virulence factors.
Additional files

**Additional file 1.** Protein identities between prophages in other Campylobacter species and C. concisus CON3965, CON3967 and CON3972. Zot proteins are in bold. Integrase proteins are underlined. \#identity: Percentage of identical amino acids (number of identical amino acids divided by number of amino acids in proteins from C. concisus 13826).

**Additional file 2.** Comparison of Campylobacter Zot proteins with known viral proteins. \#identity: Percentage of identical amino acids (number of identical amino acids divided by number of amino acids in proteins from Campylobacter species).

**Additional file 3.** Comparison of Campylobacter Zot proteins with V. cholerae Zot and N. meningitidis Zot. \#identity: percentage of identical amino acids (number of identical amino acids divided by number of amino acids of V. cholerae Zot or N. meningitidis Zot). V. cholerae Zot sequence Accession No. is AAF299647, N. meningitidis Zot sequence Accession No. is EU63554.

**Additional file 4.** Comparison of Zot proteins from Campylobacter species, N. meningitidis and V. cholerae. * indicates identical amino acids (shaded in red). \_ indicates conservative mutations (shaded in blue). . indicates semi-conservative mutations. Transmembrane domains are underlined. Walker A and walker B motifs in the N-terminus of Campylobacter Zot proteins were identified and boxed. Walker A has a sequence of GooGluG[ST], where x is any residue. Walker B motif has a sequence of hhhH[DE] where h is a hydrophobic residue [25].

Abbreviations
IBD: inflammatory bowel disease; Zot: zonula occludens toxin; ZotCampyType_1: Zot protein encoded by Cluster 1 Campylobacter; ZotCampyType_2: Zot protein encoded by Cluster 2 Campylobacter; p-loop containing nucleoside triphosphate hydrolase.

Authors’ contributions
FL and HL conducted the bioinformatics analysis. LZ conceived the project. RL wrote and provided critical feedback on bioinformatics analysis. FL, LZ, HL and RL wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The dataset supporting the conclusions of this article is included within the article (and its additional files).

Funding
This work is supported by a Faculty Research Grant awarded to Dr. Li Zhang from the University of New South Wales (Grant No: P535329).

Received: 13 August 2016 Accepted: 8 September 2016
Published online: 15 September 2016

References
1. Lastovica AJ, On SL, Zhang L. The family Campylobacteraceae—the prokaryotes. New York: Springer, 2014. p. 307–35.
2. Lee H, Ma R, Grimm MC, Riordan SM, Lan R, Zhong L, Raferty M, Zhang L. Examination of the anaerobic growth of Campylobacter concisus strains. Int J Microbiol. 2014;2014:476047.
3. Zhang L, Budiman V, Day AS, Mitchell H, Lemberg DA, Riordan SM, Grimm M, Leach ST, Ismail Y. Isolation and detection of Campylobacter concisus from saliva of healthy individuals and patients with inflammatory bowel disease. J Clin Microbiol. 2010;48:2965–7.
4. Tanner ACR, Badger S, Lai C, Listgarten MA, Visconti RA, Socransky SS. Wolinella gen. nov., Wolinella succinogenes (Wibio succinogenes Wolin et al.) comb. nov, and Description of bacteroides gracilis sp. nov., Wolinella recta sp. nov., Campylobacter concisus sp. nov., and Eikenella corrodens from humans with periodontal disease. Int J Syst Bacteriol. 1981;31(4):432–45.
5. Zhang L, Man SM, Day AS, Leach ST, Lemberg DA, Dutt S, Stormon M, Otley A, O’Loughlin EV, Magoffin A, et al. Detection and isolation of Campylobacter species other than C. jejuni from children with Crohn’s disease. J Clin Microbiol. 2009;47:453–5.
6. Mukhopadhyay I, Thomson JM, Hansen R, Berry SH, El-Omar EM, Hold GL. Detection of Campylobacter concisus and other Campylobacter species in colonic biopsies from adults with ulcerative colitis. PLoS ONE. 2011;6:e21490–1.
7. Mahendran V, Riordain SD, Grimm MC, Tran TA, Major J, Kaakouk NQ, Mitchell H, Zhang L. Prevalence of Campylobacter species in adult Crohn’s disease and the preferential colonization sites of Campylobacter species in the human intestine. PLoS ONE. 2011;6:e25417.
8. Lastovica AJ. Emerging Campylobacter spp.: the tip of the iceberg. Clin Microbiol Newsfl. 2006;28:49–56.
9. Kirk KF, Nielsen HL, Thorlacious-Usong O, Nielsen H. Optimized cultivation of Campylobacter concisus from gut mucosal biopsies in inflammatory bowel disease. Gut Pathog. 2016;8:27.
10. Zhang L. Oral Campylobacter species: initiators of a subgroup of inflammatory bowel disease? World J Gastroenterol. 2015;21:9239–44.
11. Lindblom G, Sjogren E, Hansson-Westenberg J, Kajser B. Campylobacter upsaliensis, C. sporum sporum and C. concisus as common causes of diarrhoea in Swedish children. Scand J Infect Dis. 1995;27:187–8.
12. Nielsen H, Ejlertsen T, Engberg J, Nielsen H. High incidence of Campylobacter concisus in gastroenteritis in North Jutland, Denmark: a population-based study. Clin Microbiol Infect. 2013;19:445–50.
13. Kalischuk L, Inglis G. Comparative genotypic and pathogenic examination of Campylobacter concisus isolates from diarrhoeic and non-diarrhoeic humans: BMC Microbiol. 2011;11:53.
14. Mahendran V, Tran YS, Riordan SM, Grimm MC, Day AS, Lemberg DA, Octavia S, Lan R, Zhang L. The prevalence and polymorphisms of zonula occluden toxin gene in multiple Campylobacter concisus strains isolated from saliva of patients with inflammatory bowel disease and controls. PLoS ONE. 2013;8:e75252.
15. Zhang L, Lee H, Grimm MC, Riordan SM, Day AS, Lemberg DA. Campylobacter concisus and inflammatory bowel disease. World J Gastroenterol. 2014;20:1259–67.
16. Mahendran V, Liu F, Riordan S, Grimm M, Tanaka M, Zhang L. Examination of the effects of Campylobacter concisus zonula occludens toxin on intestinal epithelial cells and macrophages. Gut Pathog. 2016;8:18.
17. Bullman S, Lucid A, Corcoran D, Sleator RD, Lucey B. Genomic investigation into strain heterogeneity and pathogenic potential of the emerging gastrointestinal pathogen Campylobacter ureolyticus. PLoS ONE. 2013;8:1.
18. Koziel M, Lucid A, Bullman S, Corcoran GD, Lucey B, Sleator RD. Draft genome sequence of Campylobacter coraciangensis strain CTF0457, a representative of a novel Campylobacter species isolated from lion-tailed macaques (Macaca silenus). Genome Announc. 2014;2:00248–314.
19. Miller WG, Yee E, On SL, Andersen LP, Bono JL. Complete genome sequence of the Campylobacter ureolyticus clinical isolate RIGS 9880. Genome Announc. 2015;3(6):01291–315.
20. Miller WG, Yee E. Complete genome sequence of Campylobacter gracilis ATCC 35236T. Genome Announc. 2015;3(6):e01087–115.
21. Miller WG, Yee E, Huysh P, Chapman MH, Parker CT. Complete genome sequence of Campylobacter gigniorum strain RM11343, isolated from an alpaca. Genome Announc. 2016;4(3):e00646–716.
22. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008;36:W5–9.
23. Mitchell A, Chang HY, Daugherty L, Fraser M, Hunter S, Lopez R, McAnulla C, McMenamin C, Nuka G, Pesset S, et al. The InterPro protein families database: the classification resource after 15 years. Nucleic Acids Res. 2015;43:D213–21.
24. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, et al. The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res. 2016;44:D279–85.

25. Hanson PI, Whiteheart SW. AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol. 2005;6:519–29.

26. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Lii WZ, Lopex R, McWilliam H, Remmert M, Soding J, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7(1):539.

27. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9.

28. Canchaya C, Proux C, Fournous G, Bruttin A, Brussow H. Prophage genomics. Microbiol Mol Biol Rev. 2003;67:238–76.

29. Pearson WR. An introduction to sequence similarity (“homology”) searching. Current Protocols in Bioinformatics. Hoboken: John Wiley & Sons Inc; 2013. p. 311–8.

30. Kaakoush NO, Man SM, Lamb S, Raftery MJ, Wilkins MR, Kovach Z, Mitchell H. The secretome of Campylobacter concisus. FEBS J. 2010;277:1606–17.

31. Fasano A, Baudry B, Pumplin DW, Wasserman SS, Tall BD, Keterly JM, Kaper JB. Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions. Proc Natl Acad Sci USA. 1991;88:5242–6.

32. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011;8:785–6.

33. Bendtsen JD, Krogh A, Sonnhammer EL. Advantages of combined transmembrane topology and signal peptide prediction-the Phobius web server. Nucleic Acids Res. 2004;32:W429–32.

34. Jackson FL, Goodman YE. Bacteroides ureolyticus, a new species to accommodate strains previously identified as “Bacteroides corrodens, anaerobic”. Int J Syst Bacteriol. 1978;28:197–200.

35. Koziel M, O'Doherty P, Vandamme P, Corcoran GD, Sleator RD, Lucey B. Campylobacter concisus sp. nov., isolated from faeces of captive lion-tailed macaques (Macaca silenus). Int J Syst Evol Microbiol. 2014;64:2878–83.

36. Miller WG, Yee E, Chapman MH. Complete genome sequences of Campylobacter hyointestinalis subsp. hyointestinalis strain LMG 9260 and C. hyointestinalis subsp. lawsonii strain LMG 15993. Genome Announc. 2016;4:00665–716.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit