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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-a 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-like-lihood 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_{CampyType_1} proteins encoded by Cluster 1 *Campylobacter zot* genes showed high similarities to each other. However, Zot_{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_{CampyType_1} proteins suggest that they are likely to have similar virulence. Zot_{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_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

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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



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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 corcagiensis*, *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 nucleo-tide 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

Table 1 Zot proteins in Campylobacter species/subspecies

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).

$Comparison \ of \ Zot_{CampyType_1} \ and \ Zot_{CampyType_2} \ proteins$

The Zot proteins encoded by Cluster 1 and Cluster 2 *Campylobacter zot* genes were referred to as $Zot_{CampyType_1}$ and $Zot_{CampyType_2}$ proteins respectively. The three $Zot_{CampyType_1}$ proteins had high similarities; they shared 171 identical amino acids and 77 conservative mutations (Fig. 3). The $Zot_{CampyType_2}$ proteins were less similar to each other as compared to $Zot_{CampyType_1}$ 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.

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	C512_RS0103935 C512_RS0100745	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 ssp. doylei	269.97	1	JJD26997_0348	Human blood	Gb0000076 ^a
C. jejuni ssp. jejuni	60004	1	CJE11_RS08060	Chicken	SAMN02429007@
C. hyointestinalis ssp. hyointestinalis	DSM 19053	1	CR67_01870	Porcine intestine	SAMN01176354@
C. hyointestinalis ssp. lawsonii	CCUG 27631	1	CHL_RS06765	Porcine intestine	[37]
C. iguaniorum	RM11343	1	CIG11343_RS03950	Alpaca faeces	[21]

^a Genome online database project ID. [@]NCBI Biosample ID

	Walker A motif	
CON_phi2_Zot2276	-MLSLIISPPRSGKTYKAVHLINDEYELHLKGESKYRFIYTNINGL	45
URE_phiZA_Zot3935	-MLTLLLGPPRSGKTYKAVNDIYEEYLKFKKNENKYRFIYTNIVGL	45
COR_phiZA_Zot6485	-MLSLILGPPRSGKTYKAVKDINDEYKKYLSNTSKYRNIYCNIGGF	45
CON_phi3_Zot0191	-MITYLI <mark>G</mark> NPGSGKT <mark>YYAVFMIYQLFLYEPKKTFLTK-FVKPKEKPNYSFCYTNINEF</mark>	56
JRE_phiZB_Zot0745	MAISYITGIPGSGKSYFAVYQIYKEFLEEPK-KKGFLNF-KKQAPKKSKYLFLYTNINQF	58
GRACI phiZ Zot2456	-MITYIVGNPGSGKTYYSVFKIYQLFIFKPKDTFLSK-VIKPEKQKEYLYCYTNINGF	56
DOYLEI phiZ Zot0348	MAITYIVGNPGSGKTYFAVNQIYEYFVLPTLPNKRILGFEIKRKLKIFDYLYCYTNINGL	60
JEJUNI phiz Zot8060	MAITYIVGNPGSGKTYFAVNQIYEYFVLPTLPNKRILGFEIKRKLKIFDYLYCYTNINGL	60
COR phiZB Zot4620	MAISYITGIPGSGKSYFAVYQIYKEFLESPK-KKGFLNF-KKQEAKKSKYEFLYTNINQF	58
HYO phiZ Zot1870	MAIHYIV SNPGSGKSYYGVYILWDKFIKQTKEPKGFLKQ-FIKPKVTKTYDIAYTNINEF	59
LAW phiz Zot6765	MAIHYIVENPGSGKSYYGVYILWDKFIKQTKEPKGFLKQFIK-PKVTKIYDIAYTNINEF	59
IGUA_phiZ_Zot3950	MSIHYIIGNPGSGKTFYGVNVLYESFIKKPKSNLLTKFIKSNDEVKKYDIAYTNINQF * * ***	58
	: : <u>* * ***:</u> : .* : . : * * ** : Walker B m <u>o</u> tif	
CON phi2 Zot2276	KFDHFDGFVKQYDKNDFLTAVSQEYTLSSQYENGFLDNVDNYDEYALKSGIYENYHHCI <mark>I</mark>	10
JRE phiZA Zot3935	KFDEFEGFVKPFNKTDFLNATIEESVLNSQHESGFLGDIADYDKYAYEKGIYKNYHHTII	10
COR phiZA Zot6485	KFELFDGFVKKFDKLDFINAVNEENLLNKQYETGFISVGNDYDSYALKNGIYEDYHHCII	10
CON phi3 Zot0191	KFELCDKFK-KFDFDEFYLGLRNLYALYKTGATDNEVNEKAKELNLYGCVF	10
JRE phiZB Zot0745	KFELKDNFI-PFDNIDFNFKLNILYQVYKSVD-GKDDTLLIEKAKELDLYQVII	11
GRACI phiZ Zot2456	KFDLDNKFI-KFDYEKFYSDLEVLYLLYMDKVGDDVLNEKAKELNLHNVII	10
OYLEI phiZ Zot0348	KFDISDKLI-SFDFDIFYLNLTHLYNLYNQGLNDDELNIKANELNLFKVMF	11
JEJUNI phiZ Zot8060	KFDISDKLI-SFDFDIFYLNLTHLYNLYNOGLNDDELNIKANELNLFKVMF	11
COR phiZB Zot4620	KFELKDNFI-PFDNTDFNFKLNQLYEVYKSTD-GKDDALLIEKAKELDLYQVII	11
IYO phiZ Zot1870	KFDKSDKII-PFDFENILSSLTILFNRYKF-E-KATDEELIKTAKQLNLLNAIF	11
LAW phiZ Zot6765	KFDKSDKII-PFDFENILSSLTILFNRYKF-E-KATDEELIKTAKQLNLLNAIF	11
IGUA phiZ Zot3950	DFTKSDKIQ-PLVFSEILSKLTLLYNEYKF-N-QASDEVLIQKSKELNLYNAIF	10
100m_pm12_20009900		10
CON phi2 Zot2276	VLDEAYNTFTKTFNDSLGRFLSYHGHFGIDIIFLFQSKRQTNREYLVHTELMYMAQPSGK	16
JRE phiZA Zot3935	VLDEAINIFIKTFNDSLGRFLSINGNEGIDITFLEQSRKQINKEILVINELMIMAQPSGK VLDEAYNTFTKEFNNSLGRFLSYHGHFGIDVVFLLQSRRQTNREYLVHTELMYMAQPSGK	16
COR phiZA Zot6485	VLDEAYNVFDKKFNDSLGRFLSYHGHFGIDVVFLLQSKRQTNREYLVHTELMYMAQPSGK	16
CON phi3 Zot0191	VLDECHNYFKNQKDEILVWWLTYHRHLYQDIYLITQDLTLVNNEYKRIAEKFYRASDSSR	16
JRE phiZB Zot0745	VLDELHNIFKNOKDEILVWWLITHKHLIGDITLITODIILVNNETKKIAEKFIKASDSSK VLDEAHNFLNDKEDEVLKWWLITHKHLYQDIILITODFSLIATGYKSIAEYFYKAIPAQL	17
	ILDEAHNFLKAKEDSILVWWLTYHRHLYQDIMLITQDISLIAIGINSIAEIFIKAIPAQL	16
GRACI_phiZ_Zot2456	VIDEAHNFLKAREDSILVWWLITHRHLHQEIIFITQDLSLISNEIRKIAEHFVKAVDSSK	10
OYLEI_phiZ_Zot0348	VIDEAHNFLKNKDDKILIWWLIIHKHLHQEIIFIIQDLSLISNEIKKIAEFFIKALDSGK VIDEAHNFLKNKDDKILIWWLTYHRHLHQEIIFIIQDLSLISNEYKRIAEFFYKALDSGK	17
JEJUNI_phiZ_Zot8060	VIDEAHNFLANKDDKILIWWLIIHKHLHQEIIFIIQDLSLISNEIKKIAEFFIKALDSGK VLDEAHNFLNDKEDEVLKWWLTYHRHLYQDIILITQDFSLIATGYKSIAEYFYKAIPAQL	17
COR_phiZB_Zot4620	VIDEIHNFFNEKENEVLIWWLTYHRHLYQELYFITQDISLIAIGINSIAEIFYRAVDSSK	17
HYO_phiZ_Zot1870	VIDEIHNFFNEKENEVLIWWLIIHKHLIQELIFIIQDLSLVNNEIKKIAEFFIKAVDSSK VIDEIHNFFNEKENEVLIWWLTYHRHLYQELYFITQDLSLVNNEYKRIAEFFYRAVDSSK	
LAW_phiZ_Zot6765		17
IGUA_phiZ_Zot3950	VIDEIHNFFNEKENEVFIWWLTYHRHLYQELYLITQDLSLVNSEYKRIAEFFYKAVDSSK ::** : :. : :*:** : :: :: *. * :* :* :* :*	16
10N		0.0
CON_phi2_Zot2276	RLFSKLFKYKVYSTSSQVNDNLINSENLKFNQKISNLYSSGSNEIYKSYATKKILFLL	22
RE_phiZA_Zot3935	RILSRLFRYKVYLTYLDYQKNYIKSENLRFNPKISNLYNSGSTKIYK <u>SYATGKIIFLL</u>	22
OR_phiZA_Zot6485	RLLSKVFRYKVYSTCDPKRDNLIKTDNIKFDSKISEIYNSGSTQIYKSYATGK <u>IFMLI</u>	22
:ON_phi3_Zot0191	RLFSKKFRYEIYASYRLFKKDRLEIINIPFLQEVFDLYHSGQSSNKKSFVR <u>FYFFLAF</u>	22
IRE_phiZB_Zot0745	RLFKNKFRYQQFSSYKLYDKDLVNRKGIHIPILPEVFALYHSGDKTSTKSFIRQ <u>LIVIGI</u>	23
RACI_phiZ_Zot2456	RLFKNKFRYMLYGSYKMYQKDVMQKFHVPYLKEVFNLYHSGQNASQKSFVRK <u>FLYVSL</u>	22
OYLEI_phiZ_Zot0348	RIYKNSLRYVQFSSYKLYQKDIVTRFSLSLNKEVFSLYKSGDNKPNKS <u>FFLKIFTFLL</u>	22
EJUNI_phiZ_Zot8060	RIYKNSLRYVQFSSYKLYQKDIVTRFSLSLNKEVFSLYKSGDNKPNKS <u>FFLKVFTFLL</u>	22
COR_phiZB_Zot4620	RLFKNKFRYQQFSSYKLFDKDLVNRKGIHIPILPEVFALYHS <mark>GDKTSQKSFIRR<u>FILIGI</u></mark>	23
IYO_phiZ_Zot1870	RFFSKKFRYIQYSNYKLYQKDIIKTFHIDFNQEIFNLYHSGQNGLGTSFVKK <u>YLFISL</u>	22
AW_phiZ_Zot6765	RFFSKKFRYIQYSNYKLYQKDIIKTFHIDFNQEIFNLYHSGQNGL <mark>GTSFVKK<u>YLFISL</u></mark>	22
[GUA_phiZ_Zot3950	RFFSKKFRYIQYSNYKLYQKDVVRTFHVDFSDECFNLYHSGKNGVGSSFVKK <u>YLLLSL</u>	22
	: :: :: : : :* ***: : .:	
	tifs in Campylobacter Zot proteins. Campylobacter Zot proteins have a transmembrane domain (underline	
	nbrane domains constitute the Zot family domains (approximately 1–210, shaded in grey) in different Car	
<i>acter</i> Zot. The Zot family domain	s belong to p-loop NTPase superfamily. Walker A and walker B motifs are in the N-terminus of Campylob	<i>acter</i> Zot

proteins. Walker A has a sequence of GxxxxGK[S/T], where x is any residue. Walker B motif has a sequence of hhhh[D/E], where h is a hydrophobic residue [25]

Prophages containing $\text{Zot}_{\text{CampyType}_1}$ 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 $Zot_{CampyType_2}$ proteins were more diverse. However,

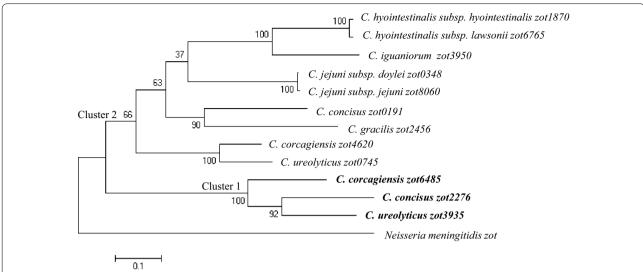


Fig. 2 The phylogenetic tree generated based on *zot* genes in different *Campylobacter* species. Maximum likelihood method was used to generate the phylogenetic tree. *Bootstrap* values were generated from 1000 replicates. Cluster 1 *zot* genes are shown in *bold*. The *zot* gene from *Neisseria meningitides* (strain 69166) was used as the outgroup

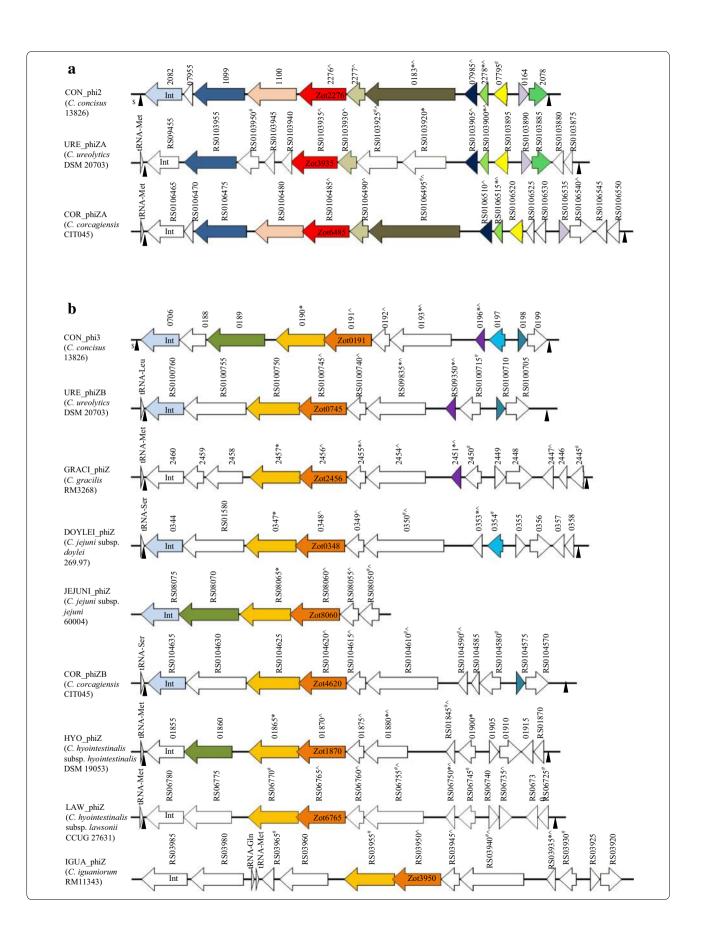
CON_phi2_Zot2276	MLSLIIGPPRSGKTYKAVHLINDEYELHLKGESKYRFIYTNINGLKFDHFDGFVKQYDKN	60
URE_phiZA_Zot3935	MLTLLLGPPRSGKTYKAVNDIYEEYLKFKKNENKYRFIYTNIVGLKFDEFEGFVKPFNKT	60
COR_phiZA_Zot6485	MLSLILGPPRSGKTYKAVKDINDEYKKYLSNTSKYRNIYCNIGGFKFELFDGFVKKFDKL	60
	** ** *********************************	
CON phi2 Zot2276	DFLTAVSQEYTLSSQYENGFLDNVDNYDEYALKSGIYENYHHCLIVLDEAYNTFTKTFND	120
URE phiZA Zot3935	DFLNATIEESVLNSQHESGFLGDIADYDKYAYEKGIYKNYHHTLIVLDEAYNTFTKEFNN	120
COR_phiZA_Zot6485	DFINAVNEENLLNKQYETGFISVGNDYDSYALKNGIYEDYHHCLIVLDEAYNVFDKKFND	120
	:.*. :* **:*.: :**.** :.***::*** ********	
CON phi2 Zot2276	SLGRFLSYHGHFGIDIIFLFQSKRQTNREYLVHTELMYMAQPSGKRLFSKLFKYKVYSTS	180
URE phiZA Zot3935	SLGRFLSYHGHFGIDVVFLLQSRRQTNREYLVHTELMYMAQPSGKRILSRLFRYKVYLTY	180
COR_phiZA_Zot6485	SLGRFLSYHGHFGID <mark>VVFLLQSKRQTNREYLVHTELMYVAQPSGKRLLSKVFR</mark> YKVYSTC	180

CON phi2 Zot2276	SQVND <mark>NLINSENLKFNQKISNLYSSGS</mark> NEIYKSYATKKILFLLAFIVFSYVVYKFLEPKH	240
URE phiZA Zot3935	LDYQK <mark>NYIKSENLRFN</mark> P <mark>KISNLYNSGSTKIYKSYATGKIIFLLLIIFISYFGYKFL</mark> KPKP	240
COR_phiZA_Zot6485	DPKRD <mark>NLIKTDNIKFD</mark> S <mark>KISEIYN<mark>SGS</mark>TQIYKSYATGK<mark>IFMLIVLAIIL</mark>YFGFKFIGP</mark> PK	240
	··* *:::*: * ***::* ***::**************	
CON phi2 Zot2276	EPAQSTKQETRFVDLNASDSKNIKAISNDADKSDINTTIFNDNKIYLRITCFPSGCKF	298
URE_phiZA_Zot3935	AKQETIIT <mark>DERFKDINRTN</mark> QDIKEPQL <mark>I</mark> Q <mark>N</mark> SDLNLDLNTT <mark>IFNDKRTYLKITC</mark> YSHF <mark>CKF</mark>	300
COR_phiZA_Zot6485	LENDKAKKDEFISEIYVSDKNQTLE <mark>I</mark> SNYKETIKDENLLLNERRI <mark>Y</mark> EKITCFPSS <mark>CKF</mark>	298
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CON phi2 Zot2276	RNYAIDLSLDSFLELLSSSNCHIFLHDKKSGNYIDYFVSCNSEFERVLKGLENSSQRVC-	357
URE_phiZA_Zot3935	RNYSLDLSLNSFLELISSFDCYIFLKDEKSANYADYYLSCPLDFSKVVSNIND-LQEIC-	358
COR_phiZA_Zot6485	RSYSLNLTLDSFLLLLADSKCSIVLTDKKSSNYIDYYVSCPAEFIGFLSKFSGDDNFYKG	358
	.:::*:**** *::* *.* *:************	
CON phi2 Zot2276	NE-KSPQTDSSSMFPTHK 374	
URE phiZA Zot3935	DE-NKGSFNTFSFK 371	
COR_phiZA_Zot6485	SQ <mark>NE</mark> NYTKNY <mark>NS</mark> FDFR 374	
	:* :: .:	
	$_{e_1}$ proteins. The protein similarities were compared using Clustal Omega. Asterisk indicates identical amino a	
(shaded in red). Colon indicates co	nservative mutations (shaded in blue). Dot indicates semi-conservative mutations. Transmembrane domains a	are

underlined

CON_phi3_Zot0191	-MITYLIGNPGSGKTYYAVFMIYQLFLYEPKKTFLTK-FVKPKEKPNYSFCYTNINEF	56
URE phiZB Zot0745	MAISYITGIPGSGKSYFAVYQIYKEFLEEPK-KKGFLNF-KKQAPKKSKYLFLYTNINQF	58
GRACI phiZ Zot2456	-MITYIVGNPGSGKTYYSVFKIYQLFIFKPKDTFLSK-VIKPEKQKEYLYCYTNINGF	56
DOYLEI phiZ Zot0348	MAITYIVGNPGSGKTYFAVNQIYEYFVLPTLPNKRILGFEIKRKLKIFDYLYCYTNINGL	60
JEJUNI_phiZ_Zot8060	MAITYIVGNPGSGKTYFAVNQIYEYFVLPTLPNKRILGFEIKRKLKIFDYLYCYTNINGL	60
COR phiZB Zot4620	MAISYITGIPGSGKSYFAVYQIYKEFLESPK-KKGFLNF-KKQEAKKSKYEFLYTNINQF	58
HYO phiZ Zot1870	MAIHYIVGNPGSGKSYYGVYILWDKFIKQTKEPKGFLKQFIK-PKVTKTYDIAYTNINEF	59
LAW phiz Zot6765	MAIHYIVGNPGSGKSYYGVYILWDKFIKOTKEPKGFLKOFIK-PKVTKIYDIAYTNINEF	59
IGUA_phiZ_Zot3950	MSIHYIIGNPGSGKTFYGVNVLYESFIKKPKSNLLTKFIKSNDEVKKYDIAYTNINQF * *: * *****:::.* ::. *: :* ** *****	58
CON_phi3_Zot0191	KFELCDKFKKFDFDEFYLGLRNLYALYKTGATDNEVNEKAKELNLYGCVFVLDECHNY	114
URE_phiZB_Zot0745	KFELKDNFIPFDNIDFNFKLNILYQVYKSVDGKDDTLLIEKAKELDLYQVLIVLDEAHNF	118
GRACI phiz Zot2456	KFDLDNKFIKFDYEKFYSDLEVLYLLYMDKVGDDVLNEKAKELNLHNVLIILDEAHNF	114
DOYLEI phiZ Zot0348	KFDISDKLISFDFDIFYLNLTHLYNLYNQGLNDDELNIKANELNLFKVMFVIDEAHNF	118
JEJUNI phiZ Zot8060	KFDISDKLISFDFDIFYLNLTHLYNLYNQGLNDDELNIKANELNLFKVMFVIDEAHNF	118
COR_phiZB_Zot4620	KFELKDNFIPFDNTDFNFKLNQLYEVYKSTDGKDDALLIEKAKELDLYQVLIVLDEAHNF	118
HYO_phiz_zot1870	KFDKSDKIIPFDFENILSSLTILFNRYKF-EKATDEELIKTAKQLNLLNAIFVIDEIHNF	118
LAW phiZ Zot6765	KFDKSDKIIPFDFENILSSLTILFNRYKF-EKATDEELIKTAKQLNLLNAIFVIDEIHNF	118
IGUA phiZ Zot3950	DFTKSDKIQPLVFSEILSKLTLLYNEYKF-NQASDEVLIQKSKELNLYNALFVIDEIHNF	117
1001_0110_0000000	.* ::: : : * *: * * : .:::*:* : ::::***	11,
CON phi3 Zot0191	FKNQKDEILVWWLTYHRHLYQDIYLITQDLTLVNNEYKRIAEKFYRASDSSRRLFSKKFR	174
URE_phiZB_Zot0745	LNDKEDEVLKWWLTYHRHLYQDIILITQDFSLIATGYKSIAEYFYKAIPAQLRLFKNKFR	178
GRACI_phiZ_Zot2456	LKAKEDSILVWWLTYHRHLYQDIMLITQDLSLISNEYKRIAEHFVKAVDSSKRLFKNKFR	174
DOYLEI phiZ Zot0348	LKNKDDKILIWWLTYHRHLHOEIIFITODLSLISNEYKRIAEFFYKALDSGKRIYKNSLR	178
JEJUNI phiz Zot8060	LKNKDDKILIWWLTYHRHLHQEIIFITQDLSLISNEYKRIAEFFYKALDSGKRIYKNSLR	178
		178
COR_phiZB_Zot4620	LNDKEDEVLKWWLTYHRHLYQDIILITQDFSLIATGYKSIAEYFYKAIPAQLRLFKNKFR	
HYO_phiZ_Zot1870	FNEKENEVLIWWLTYHRHLYQELYFITQDLSLVNNEYKRIAEFFYRAVDSSKRFFSKKFR	178
LAW phiZ Zot6765	FNEKENEVLIWWLTYHRHLYQELYFITQDLSLVNNEYKRIAEFFYRAVDSSKRFFSKKFR	178
IGUA phiZ Zot3950	FNEKENEVFIWWLTYHRHLYQELYLITQDLSLVNSEYKRIAEFFYKAVDSSKRFFSKKFR	177
	:: :.:.: *********:*:: :****::*: . ** *** *	576.0
CON phi3 Zot0191	YEIYASYRLFKKDRLEIINIPFLOEVFDLYHSGOSSNKKSFVRFYFFLAFLVFIFLLL	232
URE_phiZB_Zot0745	YQQFSSYKLYDKDLVNRKGIHIPILPEVFALYHSGDKTSTKSFIRQLIVIGIMIFILLFI	238
GRACI_phiZ_Zot2456	YMLYGSYKMYQKDVMQKFHVPYLKEVFNLYHSGQNASQKSFVRKFLYVSLFLFITLSI	232
DOYLEI_phiZ_Zot0348	YVQFSSYKLYQKDIVTRFSLSLNKEVFSLYKSGDNKPNKSFFLKIFTFLLFSILTLIF	236
JEJUNI phiz Zot8060	YVQFSSYKLYQKDIVTRFSLSLNKEVFSLYKSGDNKPNKSFFLKVFTFLLFSILTLIF	236
	YQQFSSYKLFDKDLVNRKGIHIPILPEVFALYHSGDKTSQKSFIRRFILIGILIFIVLFI	238
COR_phiZB_Zot4620		
HYO_phiZ_Zot1870	YIQYSNYKLYQKDIIKTFHIDFNQEIFNLYHSGQNGLGTSFVKKYLFISLIIFGFCIV	236
LAW phiz Zot6765	YIQYSNYKLYQKDIIKTFHIDFNQEIFNLYHSGQNGLGTSFVKKYLFISLIIFGFCII	236
IGUA phiZ Zot3950	YIQYSNYKLYQKDVVRTFHVDFSDECFNLYHSGKNGVGSSFVKKYLLLSLMIAIITAI	235
	:::. : :: * * **:****	
CON phi3 Zot0191	F <mark>FYFVVM</mark> SLFET D KPKNENLPIENKFPAPVSEQPKN-SSLFFDDKKPK	279
URE phiZE Zot0745	GFKFFINKVLLKDVPKNEPAISDQQTDLSTNDFLKPVEKNQ	279
GRACI phiz Zot2456	YFYFFVKSFNSDESA-DSSAPAPDTQSNQPIETASGNSTKALFNASNPN	280
DOYLEI_phiZ_Zot0348	CFYIFI-SFFKSDEIKENNISKESNINLNLNISPNTIKDKSENNLFSNLELIDGSLKDLP	295
JEJUNI_phiZ_Zot8060	CEYIFI-SFFKSDEIKENNISKESNINLNLNISPNTIKDKSENNLFSNLELIDGSLKDLP	295
COR phiZB Zot4620	AFKFFISNIILKDAPKDNAIKIDEKTEISNNEFLNSVNI-T	278
HYO phiZ Zot1870	AFAIFVNSITP-DTPKKDIQNSNI-QNTTELPIAKNNTFGQI	276
LAW phiz Zot6765	AFAIFVNSITP-DTPKKDIQNSNI-QNTTDTAFPITKNNTFGQI	278
	FFSIFVLYMTP-DIPENKPI-ODFNSTSKPINKP	
IGUA_phiZ_Zot3950	* :.: . : :	267
2011 1 1 2 2 4 01 01		225
CON_phi3_zot0191	NNNIDLPEIYI <mark>Y</mark> DITCLNNNCHFSDDYHLYPLSLITYISSTHTPLYFYFEPKSHELV	336
URE_phiZB_Zot0745	DLNFESKYNFV <mark>Y</mark> VFYCLKGYCNLKDEKEFYPHDIVSN I VLSSDPVYAKEISSFKNMQ	336
GRACI phiZ Zot2456	PNEPPIGYIYQIYCFYDRCSIQNGTYDHFDQRYLNFIFLRSPPKFNVRSFKGKGIT	336
DOYLEI phiZ Zot0348	LKNVDINNSSV <mark>YKILC</mark> IDTTCHIDDKNQNFMHFPLEYFHFILNEFPPIYHYKNKVNKGYQ	355
JEJUNI phiz Zot8060	LKNVDINNSSVYKILCIDTTCHIDDKNQNFMHFPLEYFHFILNEFPPIYHYKNKVNKGYQ	355
COR_phiZB_Zot4620	DQPYKNRYKFT <mark>YQFYC</mark> IKGYCNLKGEKEFLPYDIVSNIVIDSNPVYAKEVSSFKDMQ	335
HYO_phiZ_Zot1870	SKKINTSEIFY <mark>YEINC</mark> INLT <mark>C</mark> SFPNSNDKFDKRAIKFLLNQTEILYETKKYNISNVE	333
LAW phiZ Zot6765	SKKINTSEIFY <mark>YEINC</mark> INLTCSFPNSNDKFDKRAIKFLLNQTEILYETKKYNISNVE	335
IGUA phiZ Zot3950	TIKINTDDLFF <mark>YQIEC</mark> VFDDCHFLNSDQIYDKKIIKFLLNKTEIV <mark>Y</mark> QSIKYRAENLE	324
	· * : *. * : · · · ·	
CON phi3 Zot0191	KYYYVFDKPVFQNLQKNNKGVSDEKFNQIPNSSVPAIK374	
and a second	IYVYVEKDPVEDELKTKKGVSENEKDSENNSTENNEKL 374	
URE_phiZB_Zot0745	에서 가장 (프로그램) 이 방법에 대한 방법에 대한 방법이 있는 것은 이 가장 이 것이라. 이 가장 이 가	
GRACI_phiZ_Zot2456	YFFVGFDKP <mark>VF</mark> DNLKKEELNEKSSFSSAIYSK368	
DOYLEI_phiZ_Zot0348	H-FIIFNFEVFNNLKKGVLKNEKDTSFTRSLF386	
JEJUNI phiz Zot8060	H-FIIFNFE <mark>VF</mark> NNLKKGVLKNEKNTSFTRSLF386	
COR phiZB Zot4620	IYIYVFENPVFDFLKTNLQGVSDEKNSFNSSFVD-F370	
HYO phiZ Zot1870	TSIYFLKDDVFKILNIKFNDKGNTDEKDNSLFSSFGSNNTSRSNQK-379	
	그렇게 그는 것 👘 그는 것 같아요. 한 것 같아요. 한 것은 것은 것은 것이 같아요	
	TSIYFIKDDVEKTINTKENDKCNTDEKDNSTESSECONNTOPONOV-391	
LAW_phiZ_Zot6765 IGUA phiZ Zot3950	TSIYFLKDDVFKILNIKFNDKGNTDEKDNSLFSSFGSNNTSRSNQK-381 TISYFIKDDVFKVLNIKFRLSYEDKKGLTDEKTSFNSLFGSDEPKRKPNQK375	

Fig. 4 Comparison of Zot_{CampyType_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



(See figrue on previous page.)

Fig. 5 Schematic illustration of protein similarities in *zot*-containing *Campylobacter* prophages. **a** *Campylobacter* prophages containing Zot_{CampyType_1} proteins. **b** *Campylobacter* prophages containing Zot_{CampyType_2} proteins. The prophages and their host *Campylobacter* strains (*in bracket*) are listed at the left side of the figure. Proteins with more than 40 % identical amino acids with proteins in CON_phi2 or CON_phi3 were labeled with the same color. The numbers above the proteins are locus tags of the genes in the NCBI database. *Int* indicates integrase. *Asterisk* and *Hashtag* indicate proteins. *Dollar* indicates multiple insertion sites for CON_phi prophages in *C. concisus* 13826 in which only the first attachment site (for CON_phi1) overlapped with tRNA [15]. *Filled triangle* indicates attachment sites

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* gene is more prevalent in *Campylobacter* species as compared to Cluster 1 *zot* genes.

 $\text{Zot}_{\text{CampyType}_1}$ proteins, which were encoded by Cluster 1 *zot* genes, were highly similar to each other. However they were less similar to $\text{Zot}_{\text{CampyType}_2}$ 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 $\operatorname{Zot}_{\operatorname{CampyType}_1}$ in *C. concisus* encoded by $\operatorname{zot}^{808T}$ 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_{CampvType 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 additional 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. meningitis* 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	Start ^a	End ^a	Attachment gene sequence ^b	tRNA (locus_tag)
CON_phi1, CON_phi2 and CON_phi3 (C. concisus 13826) ^c	1582286	1582311	TTCAAATCCCTCTCTGTCCGCCACCA	tRNA-Ser (CCC13826_RS07905)
	1587508	1587533	TTCAAATCCCTCTCTGTCCGCCACCA	
	1597113	1597138	TTCAAATCCCTCTCTGTCCGCCACCA	
	1606718	1606743	TTCAAATCCCTCTCTGTCCGCCACCA	
	1616160	1616185	TTCAAATCCCTCTCTGTCCGCCACCA	
CON_phi4 (<i>C. concisus</i> 13826)	946941	946993	CTCATAACCCGAAGGTCGGCGGTTCAA ATCCGTCCTCCGCAACCAAATACCGA	tRNA-Met (CCC13826_RS04780)
	937290	937342	CTCATAACCCGAAGGTCGGCGGTTCAA ATCCGTCCTCCGCAACCAAATACCGA	
URE_phiZA (C. ureolyticus	68067	68122	ATAACCCGAAGGTCGGAGGTTCAAGTCCT TCCTCTGCAACCAAATCACCATTTTAC	tRNA-Met (C512_RS0103965)
DSM 20703)	57811	57866	ATAACCCGAAGGTCGGAGGTTCAAGTCCT TCCTCTGCAACCAAATCACCATTTTAC	
URE_phiZB (C. ureolyticus DSM 20703)	139564	139610	<i>GTTCAAGTCTCGCTGATCGCACCA</i> TTA AAGAAAAAATTAAGAATACT	tRNA-Leu (C512_RS0100765)
	130103	130149	GTTCAAGTCTCGCTGATCGCACCATTA AAGAAAAAATTAAGAATACT	
COR_phiZA (C. corcagiensis	254051	254088	CGAAGGTCAGGGGTTCAAGTCCCTTCT CTGCAACCAAA	tRNA-Met (SA94_RS06290)
CIT045)	265302	265339	CGAAGGTCAGGGGTTCAAGTCCCTTCT CTGCAACCAAA	
COR_phiZB (C. corcagiensis	257240	257264	GTTCAAATCCCTCTCTGTCCGCCAC	tRNA-Ser (SA94_RS04510)
CIT045)	247319	247343	GTTCAAATCCCTCTCTGTCCGCCAC	
GRACI_phiZ (C. gracilis	112826	112871	CTCATAACCCGAAGGTCGGTGGTTCAA ATCCACCCTCTGCAACCAA	tRNA-Met (CAMGR0001_2931)
RM3268)	102518	102563	CTCATAACCCGAAGGTCGGTGGTTCAA ATCCACCCTCTGCAACCAA	
DOYLEI_phiZ	303215	303241	AGGGTTCAAATCCCTCTCTGTCCGCCA	tRNA-Ser (JJD26997_RS01570)
(C. jejuni subsp. doylei 269.97)	313165	313191	AGGGTTCAAATCCCTCTCTGTCCGCCA	
HYO_phiZ (<i>C. hyointestinalis</i> subsp. <i>hyointestinalis</i> DSM 19053)	360409	360446	CTCATAACCCGAAGGTCGGAGGTTCAA GTCCTTCTCTC	tRNA-Met (CR67_RS01810)
	369716	369753	CTCATAACCCGAAGGTCGGAGGTTCAA GTCCTTCTCTC	
HYO_phiZ (C. <i>hyointestinalis</i> subsp. <i>lawsonii</i> CCUG 27631)	1283134	1283238	<i>CTCATAACCCGAAGGTCGGAGGTTCAAGT CCTTCTCTCGCAACCA</i> AATAAGCATAAAA TCATCTTTTAAAGCACATTGTTTTAAAGCT TAAAATAATCTTACTTT	tRNA-Met (CHL_RS06785)
	1273720	1273824	CTCATAACCCGAAGGTCGGAGGTTCAAGT CCTTCTCTCGCAACCAAATAAGCATAAAA TCATCTTTTAAAGCACATTGTTTTAAAGCT TAAAATAATCTTACTTT	

^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 JEJUNI_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 $Zot_{CampyType_1}$ proteins suggest that they are likely to have similar virulence. $Zot_{CampyType_2}$ 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 *Campy-lobacter* species and *C. concisus* CON_phi2 and CON_phi3. 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* prophage 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 AAF29547. *N. meningitidis* Zot sequence Accession No. is EJU63554.

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 GxxxrGK[S/T], where x is any residue. Walker B motif has a sequence of hhhh[D/E], where h is a hydrophobic residue [25].

Abbreviations

IBD: inflammatory bowel disease; Zot: zonula occludens toxin; Zot_{CampyType_1}: *Campylopbacter* Zot protein encoded by Cluster 1 *zot* gene; Zot_{CampyType_2}: *Campylopbacter* Zot protein encoded by Cluster 2 *zot* gene; p-loop NTPase: p-loop containing nucleoside triphosphate hydrolase.

Authors' contributions

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

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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).

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References

- 1. Lastovica AJ, On SL, Zhang L. The family Campylobacteraceae—the prokaryotes. New York: Springer; 2014. p. 307–35.
- Lee H, Ma R, Grimm MC, Riordan SM, Lan R, Zhong L, Raftery 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., WoZinella succinogenes (Vibrio 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.
- 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.
- Mukhopadhya 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.
- Mahendran V, Riordan SM, Grimm MC, Tran TA, Major J, Kaakoush NO, 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.
- Lastovica AJ. Emerging *Campylobacter* spp.: the tip of the iceberg. Clin Microbiol Newsl. 2006;28:49–56.
- Kirk KF, Nielsen HL, Thorlacius-Ussing 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.
- Lindblom G, Sjogren E, Hansson-Westerberg J, Kaijser B. Campylobacter upsaliensis, C. sputorum sputorum and C. concisus as common causes of diarrhoea in Swedish children. Scand J Infect Dis. 1995;27:187–8.
- Nielsen H, Ejlertsen T, Engberg J, Nielsen H. High incidence of *Campylo-bacter concisus* in gastroenteritis in North Jutland, Denmark: a population-based study. Clin Microbiol Infect. 2013;19:445–50.
- Kalischuk L, Inglis G. Comparative genotypic and pathogenic examination of *Campylobacter concisus* isolates from diarrheic and non-diarrheic humans. BMC Microbiol. 2011;11:53.
- Mahendran V, Tan 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:e75525.
- 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.
- 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.
- 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.
- Koziel M, Lucid A, Bullman S, Corcoran GD, Lucey B, Sleator RD. Draft genome sequence of *Campylobacter corcagiensis* strain CIT045T, a representative of a novel *Campylobacter* species isolated from lion-tailed *macaques* (*Macaca silenus*). Genome Announc. 2014;2:00248–314.
- 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.
- Miller WG, Yee E. Complete genome sequence of *Campylobacter gracilis* ATCC 33236T. Genome Announc. 2015;3(6):e01087–115.
- Miller WG, Yee E, Huynh S, Chapman MH, Parker CT. Complete genome sequence of *Campylobacter iguaniorum* strain RM11343, isolated from an alpaca. Genome Announc. 2016;4(3):646–716.
- 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.
- Mitchell A, Chang HY, Daugherty L, Fraser M, Hunter S, Lopez R, McAnulla C, McMenamin C, Nuka G, Pesseat S, et al. The InterPro protein families database: the classification resource after 15 years. Nucleic Acids Res. 2015;43:D213–21.

- 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.
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Liii WZ, Lopez R, McWilliam H, Remmert M, Soding J, et al. Fast, scalable generation of highquality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7(1):539.
- 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.
- Canchaya C, Proux C, Fournous G, Bruttin A, Brussow H. Prophage genomics. Microbiol Mol Biol Rev. 2003;67:238–76.
- Pearson WR. An introduction to sequence similarity ("homology") searching. Current Protocols in Bioinformatics. Hoboken: John Wiley & Sons Inc; 2013. p. 311–8.
- 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.
- Fasano A, Baudry B, Pumplin DW, Wasserman SS, Tall BD, Ketley JM, Kaper JB. Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions. Proc Natl Acad Sci USA. 1991;88:5242–6.

- Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011;8:785–6.
- Bendtsen JD, Kiemer L, Fausboll A, Brunak S. Non-classical protein secretion in bacteria. BMC Microbiol. 2005;5:58.
- 34. Kall L, Krogh A, Sonnhammer EL. Advantages of combined transmembrane topology and signal peptide prediction-the Phobius web server. Nucleic Acids Res. 2007;35:W429–32.
- 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.
- Koziel M, O'Doherty P, Vandamme P, Corcoran GD, Sleator RD, Lucey B. Campylobacter corcagiensis sp. nov., isolated from faeces of captive lion-tailed macaques (*Macaca silenus*). Int J Syst Evol Microbiol. 2014;64:2878–83.
- 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.

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