Title: *Lactobacillus fermentum* 3872 as a potential tool for combatting *Campylobacter jejuni* infections

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Abstract

Due to the global spread of multidrug resistant pathogenic bacteria, alternative approaches in combating infectious diseases are required. One such approach is the use of probiotics. *Lactobacillus fermentum* 3872 is a promising probiotic bacterium producing a range of antimicrobial compounds, such as hydrogen peroxide and lactic acid. In addition, previous studies involving genome sequencing and analysis of *L. fermentum* 3872 allowed the identification of a gene encoding a collagen binding protein (CBP) not found in any other species of this genus. In this study, we found that the CBP of *L. fermentum* 3872 binds to collagen I present on the surface of the epithelial cells lining the gastrointestinal tract. Moreover, we found that this host receptor is also used for attachment by the major gastrointestinal pathogen, *Campylobacter jejuni*. Furthermore, we identified an adhesin involved in such interaction and demonstrated that both *Lactobacillus fermentum* 3872 and its CBP can inhibit binding of this pathogen to collagen. Combined with the observation that *C. jejuni* growth is affected in the acidic environment produced by *L. fermentum* 3872, the finding provides a good basis for further investigation of this strain as a potential tool for fighting Campylobacter infections.

Introduction

Campylobacter jejuni is an enteric pathogen and one of the most common causes of gastroenteritis in humans with symptoms such as abdominal pains, watery or bloody diarrhoea, and fever [1]. In rare cases, *C. jejuni* infections can lead to a neurodegenerative disease such as Guillain-Barre syndrome [2]. *C. jejuni* infections are often caused by poor hygiene standards, consumption of undercooked meat, contaminated water and/or milk [3]. Fatalities associated with *C. jejuni* infections are uncommon, although can occur among immunologically naïve patients [4]. *C. jejuni* infections are an economic burden leading to many hospitalisations/primary care visits [5].

There has been a rise in antimicrobial resistant forms of *C. jejuni* caused by the misuse of antimicrobials [6]. *C. jejuni* has also been placed on a list of antibiotic-resistant priority pathogens by the world health organisation (WHO) to promote research and development in novel antimicrobials [7]. Due to the appearance of multidrug resistant forms of these bacteria, there is growing interest in using alternative approaches to combat *C. jejuni* infections. Among the antimicrobial factors being considered are probiotics, bacteriocins and bacteriophages, with the most recent focus on probiotics [1]. Effective usage of the latter requires a better understanding of the molecular mechanisms of their action. The antibacterial activity of probiotics is associated with the production of bacteriocins, lactic acid, hydrogen peroxide, competition for nutrients and colonisation niches, as well as modulation of host immune response [1, 8].

Lactobacillus fermentum 3872 is a Gram-positive, facultative anaerobe isolated from a healthy human female [9]. *L. fermentum* 3872 produces lactic acid and hydrogen peroxide and is capable of binding to host cells[9]. Genome sequencing of *L. fermentum* 3872 revealed the genes required for bacterial survival in the gastrointestinal tract, as well as those potentially involved in the attachment to fibronectin, mucin, and collagen. The full and partial copies of

the collagen-binding protein (CBP) encoding genes were found to be located on a plasmid and chromosome respectively [10, 11]. Collagen I, which is one of several types of collagens ubiquitous in mammals, is commonly found on the surface of the host cells present in the gastrointestinal tract [12]. In this study, we confirmed the affinity of the putative CBP of *L. fermentum* 3872 to collagen I and found that both CBP and *L. fermentum* 3872 compete with *C. jejuni* for binding to this host cell receptor. In addition, a role of *C. jejuni* flagellum in binding to collagen I was established.

Results

The purified CBP of *L. fermentum* 3872 interferes with *Campylobacter* binding to collagen 1

Expression of the *L. fermentum* 3872 CPB in *E. coli* as a His-tagged fusion protein allowed its purification as a stable product of an expected size (111 kDa predicted, 115 kDa estimated from a gel, Fig. S1). A slight (3.6%) difference in the sizes is likely to be due to conformational properties of this protein. Abnormal migration of large outer membrane proteins on SDS gels, has been reported previously [13]. It was found that the CBP binds to collagen I in a concentration-dependent manner with saturation with concentrations above 0.1 µg/well of CBP (Fig. 1). Since *C. jejuni* strains 11168H and 81-176 were also able to bind to collagen I in a concentration-dependent manner (Fig. 2), we aimed to establish if these bacteria compete with the purified CBP for the binding sites.

Inhibition of *C. jejuni* attachment to collagen I was indeed confirmed when using 2 µg of CBP per well (Fig. 3). We were then wondering if a similar inhibition of *Campylobacter* could be observed when using whole cells of *L. fermentum*. The inhibition was confirmed when using high *Lactobacillus*/*Campylobacter* cell ratios (Fig. 4). Surprisingly, some increase in *C. jejuni* binding was seen when using smaller amounts of *L. fermentum* 3872

(Fig. 4). As described in the discussion section, this could be a result of aggregation of *Campylobacter* bacteria.

Identification of *C. jejuni* proteins involved in collagen I binding

Whilst genome sequencing reveals genes encoding potential collagen binding proteins of *L. fermentum* 3872, one of which was the subject of this study, no such proteins could be identified in the genomes of *C. jejuni* strains 11168H and 81-176. Therefore, attempts to identify such proteins were undertaken by using affinity binding followed by mass spectrometry (LC MS/MS). The analysis of the proteins bound to magnetic beads coated with collagen I revealed 2 major bands (66 kDa and 14 kDa, Fig. 5) in both strains tested Analysis of these bands using mass spectrometry identified the top (approximately 66 kDa) bands in both strains as flagellin subunits (FlaA and FlaB). Larger observed sizes of these proteins, when compared to those predicted from their amino acid sequences (60 kDa), are likely to be a result of O-linked glycosylation [14]. Indeed, it was reported that the molecular mass of flagellin of strain 81-176 as determined by gel electrophoresis was about 6 kDa larger than that was predicted from its amino acid sequence [15]. Slight difference in gel mobilities between flagellins from these two strains (Fig. 5) can also be explained by strain to strain variation in glycosylation pattern. According to LC MS/MS analysis, the lower bands (ca 14 kDa) seen appeared to be FlaA and FlaB fragmentation products.

***L. fermentum* 3872 inhibits *C. jejuni* growth by production of acidic environment**

Cell-free supernatants of *L. fermentum* inhibited the growth of *C. jejuni* (Fig. 6). Adjustment of the supernatant pH (normally about 4.2) to 6.3 abolished inhibition zones, suggesting that it was the acid environment that was causing the inhibitory effect. Acidification of the media is commonly attributed to the release of lactic acid by these bacteria. On the other hand, heat

treatment had no effect on the inhibition zone (Fig. 6) indicating the absence of heat labile compounds (proteins) involved in growth inhibition. The results suggest that the main anti-*Campylobacter* activity is associated with acidification of the environment.

Discussion and conclusion

The results of this study suggest that *L. fermentum*) (probiotic) and *Campylobacter jejuni* (pathogen) may exploit the same host cell receptor for attachment and colonisation. We demonstrated the molecular mechanism of such interaction and identified the adhesins required for binding of these bacteria to collagen receptor. In addition, we provided a proof of principle for the development of antibacterial tools based on the inhibition of pathogen binding to host cells in the presence of probiotics via competitive exclusion. As adhesion is important for *C. jejuni* host colonisation and infection [16], competition for adhesion to collagen I may be a viable means of reducing pathogen load in hosts and thus preventing *C. jejuni* infection. Interestingly, whole cell ELISA experiments indicated more *C. jejuni* being detected when using lower amounts of *L. fermentum* 3872. This could be explained by possible auto-aggregation of *C. jejuni* or co-aggregation between *C. jejuni* and *L. fermentum* 3872. Co-aggregation between *C. jejuni* and other species of *Lactobacilli* had previously been reported [17]. In a study conducted by Nishiyama *et al.* it was observed that the treatment of chicks with *L. gasseri* LG2005 over a period of 14 days resulted in reduced colonisation of birds by *C. jejuni* 81-176 [18]. In addition, *in vitro* experiments demonstrated that the presence of probiotics can lead to co-aggregation with *C. jejuni* and inhibition of adhesion of the latter to human epithelial cells [18].

The ability of probiotic bacteria to cause aggregation of (or co-aggregation with) *C. jejuni* cells and inhibit their binding to host cells may work synergistically with other antibacterial factors. In particular, higher gastric acidity was found to reduce the likelihood of *C. jejuni*

infection [19]. This is supported by our study demonstrating that acidification of the environment caused by *Lactobacillus fermentum* represents a strong antibacterial factor. Co-aggregation may assist in antibacterial action of lactobacilli by reducing the distance between the probiotic cells and the pathogen, thus increasing the local concentration of the antibacterial compounds [17]. Utilisation of multiple antibacterial factors would elevate antibacterial activity of probiotics and reduce the risk of development of antimicrobial resistance in pathogenic bacteria.

According to our results, *C. jejuni* flagellin binds to collagen I, supporting other data on the role of flagella in adhesion [20, 21]. To our knowledge, this is the first study on the identification of a host cell molecule specifically interacting with bacterial flagellum. The latter is known to be modified by O-linked glycosylation, which is variable both within the same strain and between different strains [22-24]. Due to extreme variability of O-linked flagella modifications and the difference between the oligosaccharide structures decorating flagellins in the two strains tested, the involvement of sugar residues in binding to collagen seems unlikely.

It would be interesting to investigate other putative *L. fermentum* 3872 adhesins predicted from its genome sequence [11], such as enolase, mucus and fibronectin binding proteins, as well as aggregation substance precursor. The results of this study warrant further investigation of antibacterial activity of this strain in poultry. Due to its anti-campylobacter activity *L. fermentum* 3872 could potentially be used for prophylaxis of such *C. jejuni* induced diseases as traveller's diarrhoea, inflammatory bowel disease and irritable bowel syndrome [25 26]. Although it was isolated from human milk of a healthy person [9] and is predicted to be generally safe, trial experiments are required to confirm its safety. The experiments described in this study may also be conducted with other pathogenic bacteria, such as e.g. *Staphylococci* which utilise adhesion to collagen for host colonisation [27]. With increasing understanding of the mechanisms of interaction and competition between bacteria, a wide

variety of tools may be developed for anti-microbial purposes, reducing our dependence on antibiotics and widening our means in combatting pathogenic bacteria such as *C. jejuni*.

Materials and methods

Bacterial strains and growth conditions

L. fermentum 3872 was grown overnight at 37°C under anaerobic conditions on de Man, Rogosa and Sharpe (M.R.S.) agar (Oxoid), and in M.R.S broth (Oxoid). *C. jejuni* 11168H is a hypermotile derivative of *C. jejuni* NCTC 11168 originally isolated from human faeces [22]. *C. jejuni* 81-176 is a highly virulent strain isolated from raw milk [28]**.** *C. jejuni* was grown for 24 hours at 37°C in a microaerobic incubator (Don Whitley Scientific) in an atmosphere of 10% CO_2, 5% O_2, N_2 85% on CBA (Columbia Blood Agar Base, Oxoid) supplemented with 5% defibrinated horse blood (Oxoid) and *Campylobacter* selective supplement Skirrow (Oxoid). *E. coli* was grown at 37°C overnight on LB (Luria Bertani) agar (Fisher Scientific) or in LB broth (Fisher Scientific) supplemented with chloramphenicol at 25 µg/ml where appropriate, e.g. for expression of CBP (see below).

Cloning and purification of CBP

The *cbp* gene lacking the region corresponding to the leader peptide was PCR-amplified using the following primers: CBP_Forward, TGCTTCTAGAAGAAGGAGGCAACAGTATGCACCATCACCATCACCATGATAGCA AGACAAATATTACTCAGAACGG and CBP_Reverse, ATGAGCATGCTCAAATAGTAAATCTACTTATAACTACTAAACC. The CBP_Forward primer contained a Shine-Dalgarno (SD) sequence, as well as a region encoding a hexa histidine tag. Polymerase chain reaction (PCR) was conducted by using a Q5 High-Fidelity DNA Polymerase kit (NEB) with the following conditions: 98°C for 30 seconds for initial

denaturation, 25 cycles of denaturation for 10 seconds at 98°C, annealing for 30 seconds at 55°C and extension for 4 minutes at 55°C, and a final extension at 72°C for 2 minutes. The PCR product was purified using the QIAquick PCR purification kit (Qiagen), digested with enzymes *Xba*I and *Sph*I (NEB) and cloned into expression vector pBAD33 [29] using Quick Ligation kit (NEB) and *E. coli* Express competent cells (NEB). Sanger sequencing, conducted by GENEWIZ, revealed no errors in the cloned fragment. For protein expression, 10 ml of the overnight culture of bacteria containing the recombinant plasmid were inoculated into 250 ml of media, incubated at 37°C on a shaker at 120 rpm to OD_{600} of 0.6 and induced with L-arabinose (ACROS organics) at a final concentration of 0.1% for 3 hours. The protein was purified using a Clonetech His60 protein purification column. The concentration of protein was determined using a Pierce BCA protein assay kit. Samples were analysed on NuPAGE Novex 4%-12% Bis-tris gel (ThermoFisher Scientific) after mixing with1X NuPAGE LDS sample buffer (ThermoFisher Scientific) and incubation at 70°C for 10 minutes, as recommended by the manufacturer. Electrophoresis was conducted using 1X NuPAGE MOPS SDS running buffer (ThermoFisher Scientific) for 1 hour at 150 V. The samples were stained using Invitrogen Coomassie Simply Blue Safe stain (ThermoFisher Scientific). Equivalent amounts of samples in relation to the number of cells were loaded onto each well.

Silver staining was conducted using the Pierce silver stain kit for mass spectrometry (ThermoFisher Scientific) according to the standard manufacturer's protocol.

The molecular marker used for Coomassie staining was PageRuler Plus Prestained protein ladder (ThermoFisher Scientific) diluted to 1:10 in 1X NuPAGE LDS buffer (ThermoFisher Scientific). For Silver staining the PageRuler Plus Prestained protein ladder (ThermoFisher Scientific) was diluted to 1:100 in 1X NuPAGE LDS buffer (ThermoFisher Scientific).

Enzyme-linked immunosorbent assay

Calf skin collagen I (Sigma) was dissolved in 0.1M acetic acid (Fisher Scientific) to a stock concentration of 1 mg/ml. Transparent Corning Costar 96 well flat bottom non-treated polystyrene plates were coated with 0.36 µg/well calf skin collagen I (Sigma) or BSA (Sigma) in ELISA coating buffer (0.19g Na_2CO_3, 0.37g $NaHCO_3$ in 125 ml dH_2O; pH 9.6). The plates were incubated at 4°C overnight. For washing steps, 200 µl/well PBS with 0.1%Tween 20 (PBST) was used. After coating, the plates were washed twice with PBST and blocked for 1 hour at room temperature with 2% BSA (Sigma) in PBS (200 µl/well). Wells were washed three times with PBST and 100 µl/well of samples were added to each well. The plates were incubated at 37°C for 1 hour. If bacteria were used, the samples were incubated under anaerobic conditions. Wells were washed four times with PBST. One hundred microliters of the primary antibody (1:1000 dilution in PBS containing 0.05% Tween20 (Sigma) and 1 mg/ml BSA (Sigma)) were added to each well and the plates were incubated at 37°C for 1 hour. Wells were washed four times with PBST and 100 µl of an appropriate secondary antibody (1:1000 dilution in PBS containing 0.05% tween 20 (Sigma) and 3% BSA (Sigma)) was added, followed by incubation for 1 hour at 37°C. Wells were washed four times with PBST and incubated with a 100 µl of 3,3′,5,5′-Tetramethylbenzidine substrate (Sigma) for 15 minutes. Fifty µl/well of 1M H_2SO_4 were added to stop the reaction. Absorbance was measured at 450 nm using a Tecan Infinite M200 Pro microplate reader.

For *C. jejuni* binding and CBP/*C.jejuni* competition assays, *Campylobacter jejuni* monoclonal primary antibody (Bio-Rad) and goat anti-mouse polyclonal secondary antibody (Bio-Rad) were used.

For CBP binding assay, Pierce 6x-His Epitope Tag monoclonal primary antibody (ThermoFisher Scientific), and anti-mouse IgG, HRP-linked Polyclonal secondary antibody (Cell Signaling Technology) were used.

For whole cell competition assay anti-*Campylobacter jejuni* (PEB1), polyclonal primary antibody (Antibodies-Online) and Goat-anti-rabbit IgG polyclonal secondary antibody HRP conjugate (SAB) were used.

Binding and competition assay

For binding assay, CBP stock was diluted in PBS. The samples were added to collagen I coated wells. BSA-coated wells were used as negative controls.

For *C. jejuni* attachment studies the wells coated with collagen I were incubated with a 100 µl of bacterial suspensions made to an OD_{600} of 1, 0.1 and 0.01 in PBS. The final amounts of *C. jejuni* cells added to each well were $2x10^8$ cfu/well, $2x10^7$ cfu/well and $2x10^6$ cfu/well respectively. BSA-coated wells were used as negative controls.

For whole cell competition assay, *L. fermentum* 3872 bacterial suspension was made to an OD_{600} of 0.5 ($1x10^8$ cfu/ml), 1 ($2x10^8$ cfu/ml), 5 ($1x10^9$ cfu/ml), and 9 ($2x10^9$ cfu/ml) by mixing with *C. jejuni* to have a final bacterial suspension of OD_{600} 0.1 ($2x10^8$ cfu/ml) in PBS. A hundred microliters of the mixture was added to each well. This resulted in a final ratio of *L. fermentum* 3872 to *C. jejuni* of 1:2, 1:1, 5:1 and 10:1 respectively.

For competition assays involving CBP and *C. jejuni*, collagen I or BSA coated wells were incubated with a mixture of 2 µg/well CBP and $2x10^7$ cfu/well *C. jejuni* in PBS.

Agar well diffusion assay

Agar well diffusion assay was used to determine anti-*C. jejuni* activity [18]. *C. jejuni* suspensions were adjusted to an OD_{600} of 1 in PBS, of which 300 µl were added to 15 ml of soft (0.75%) Mueller-Hinton (MH) agar at 41°C. Soft agar was prepared by mixing MH broth (Fluka) to 0.75% agar (Fluka). The inoculated molten agar was overlaid over 20 ml MH agar. *L. fermentum* 3872 was cultured overnight in M.R.S broth at 37°C under anaerobic condition

and filter sterilised using a 0.22 µm filter (Fisher Scientific). The cell-free culture supernatant was either boiled at 100°C for 5 minutes, or the pH was adjusted to that of the M.R.S broth (6.3) using NaOH (Sigma). Four 10 mm wells were cut in the MH agar after inoculating with *C. jejuni*. The wells were filled with 300 µl of one of the following 1) MH broth, 2) cell-free 3872 culture supernatant 3) boiled cell-free 3872 culture supernatant, or 4) cell-free 3872 culture supernatant with adjusted pH.

Co-Immunoprecipitation and Mass Spectrometry

Co-Immunoprecipitation (Co-IP) was conducted using Dynabeads Co-Immunoprecipitation kit (ThermoFisher Scientific) to determine collagen I binding proteins expressed by *C. jejuni* 11168H and 81-176. Dynabeads were coated with 15 µg of collagen I (15 µg of collagen per 1 mg of Dynabeads). Standard manufacturer's protocol was followed.

C. jejuni suspension was made to an OD_{600} of 1 in 20 ml of PBS. The cell suspension was spun down at 3200 g for 10 minutes at 4°C, the supernatant was removed and the bacteria pellet was weighed. Lysis buffer was prepared using 1X IP buffer provided by the Dynabeads Co-Immunoprecipitation kit (ThermoFisher Scientific), 100mM NaCl (Sigma) and 5 µl DNase I (Promega) in dH_2O. The bacterial pellet was lysed in a 1:9 ratio of cell weight to volume in a lysis buffer, and incubated on ice for 10 minutes. To ensure complete lysis, bacterial lysates were sonicated (Soniprep 159) for 10 cycles with 10 seconds sonication and 30 seconds rest, to complete lysis. After sonication, the samples were spun at 3200 g for 5 minutes. The clarified lysates were incubated at room temperature with 2 mg of collagen I coupled Dynabeads on a rotator (30 rpm) for 1 and 3 hours. Standard manufacturer's protocol was followed for protein elution. Prior to mass spectrometry, Pierce silver stain kit (ThermoFisher Scientific) was used to detect protein bands (see above). Bands were cut out using a scalpel and placed into an Eppendorf tube with 50 µl of dH_2O. Mass spectrometry

was conducted by the Cambridge Centre for Proteomics (United Kingdom). Mascot server was used for the identification of proteins using Genbank data of the respective *C. jejuni* strains.

Statistical analysis

ELISA readings were adjusted by subtracting values of relevant controls. Statistical analysis was conducted using one-way analysis of variance (ANOVA). A P value of <0.05 was considered as statistically significant. On ELISA graphs the P variances are labelled by stars according to the following scheme: * for $0.005<p\leq0.05$, ** for $0.001<p\leq0.005$ and *** for $p\leq0.001$. The vertical bars on the diagrams represent SEMs (standard errors of the mean).

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Figure legends

Figure 1

ELISA experiments showing CBP binding to collagen I; the data represent two biological repeats each with three technical repeats (n=6).

Figure 2

Detection of adhesion of *C. jejuni* strains 81-176 (A) and 11168H (B) to collagen I using ELISA; the data represent two biological repeats each with three technical repeats (n=6) apart from (A), $2x10^8$ cfu/well, where n=5.

Figure 3

Effect of CBP (2 µg/well) on adhesion of *C. jejuni* strains 81-176 (A) and 11168H (B) in binding to collagen I, the bars labelled 'control' have respective *C. jejuni* strains ($2x10^7$ cfu/well) only, while that labelled CBP has a mixture of 2 µg CBP and $2x10^7$ cfu/well *C. jejuni*; the data represent three biological repeats each with three technical repeats (n=9).

Figure 4

Competition between *L. fermentum* 3872 and *C. jejuni* strains 81-176 (A) and 11168H (B) for binding to collagen I detected using ELISA, the bars labelled 'control' have respective *C. jejuni* strains of amount $2x10^7$ cfu/well added only, the ratios indicated on the graph are based on cell to cell amounts added to each well; the data represent two biological repeats each with three technical repeats (n=6). Control (B) and 1:10 (B) represent data with three biological repeats each with three technical repeats each (n=9).

Figure 5

Inhibition of growth of *C. jejuni* strains 81-176 (A) and 11168H (B) in the presence of cell-free *L. fermentum* 3872 culture supernatant; 1, M.R.S broth (control); 2-4, *L. fermentum* 3872 cell-free supernatant; 2, untreated; 3, heat-treated; 4, pH adjusted. Three biological repeats were carried out.

Figure 6

Silver staining of *C. jejuni* Co-IP eluate; 1, pre-stained ladder (Page ruler plus); 2, 11168H eluate after 1 hour incubation; 3, 11168H eluate after 3 hour incubation; 4, 81-176 eluate after 1 hour incubation; 5, 81-176 eluate after 3 hour incubation.

Figure S1

Coomassie staining of the recombinant CBP: 1, lysate before induction; 2, lysate after 3 hours induction; 3, clarified lysate; 4, flowthrough; 5, wash; 6, eluate 1; 7, eluate 2; 8, eluate 3; 9, pre-stained ladder (Page ruler plus).

Figure S2

Screenshots of Mascot analysis of LC MS/MS output data; the diagram shows predominant hits for a 65 kDa collagen binding protein detected in *C. jejuni* strains 81-176 (A), 11168H (B).

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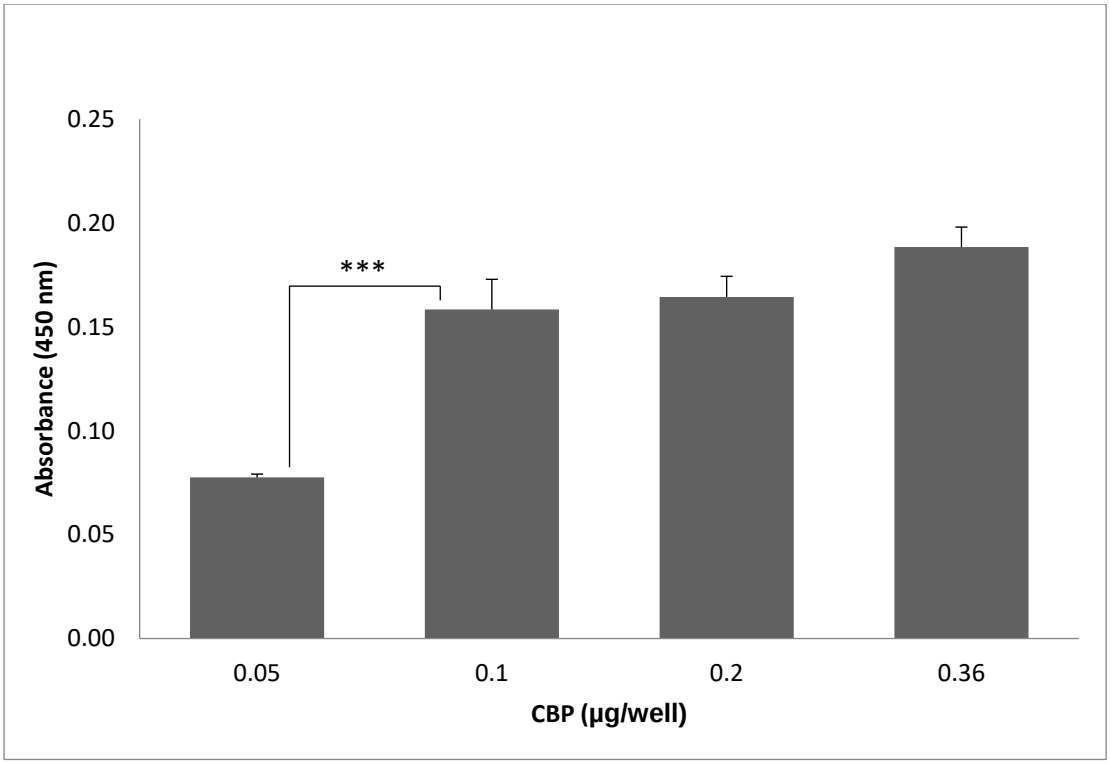


Figure 1

ELISA experiments showing CBP binding to collagen I; the data represent two biological repeats each with three technical repeats (n=6).

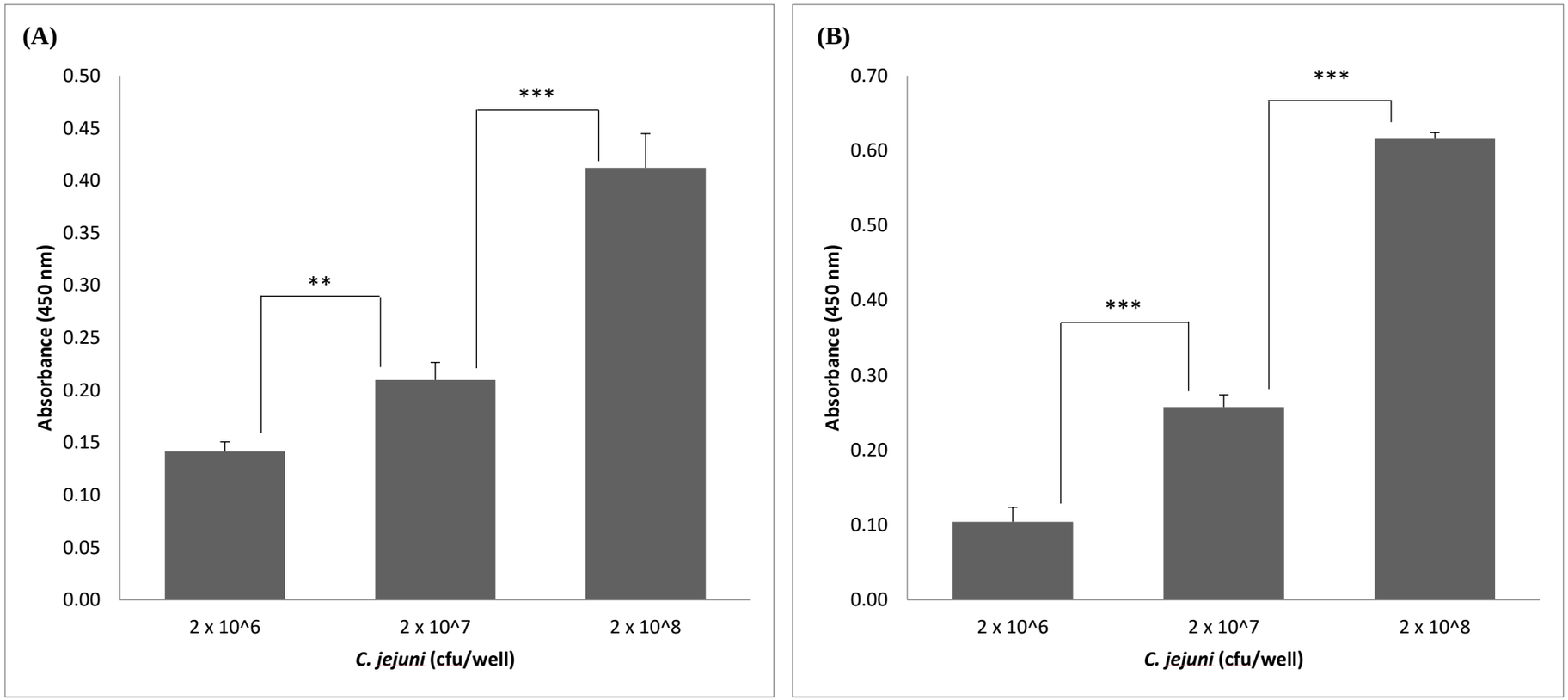


Figure 2

Detection of adhesion of *C. jejuni* strains 81-176 (A) and 11168H (B) to collagen I using ELISA; the data represent two biological repeats each with three technical repeats (n=6) apart from (A), $2x10^8$ cfu/well, where n=5.

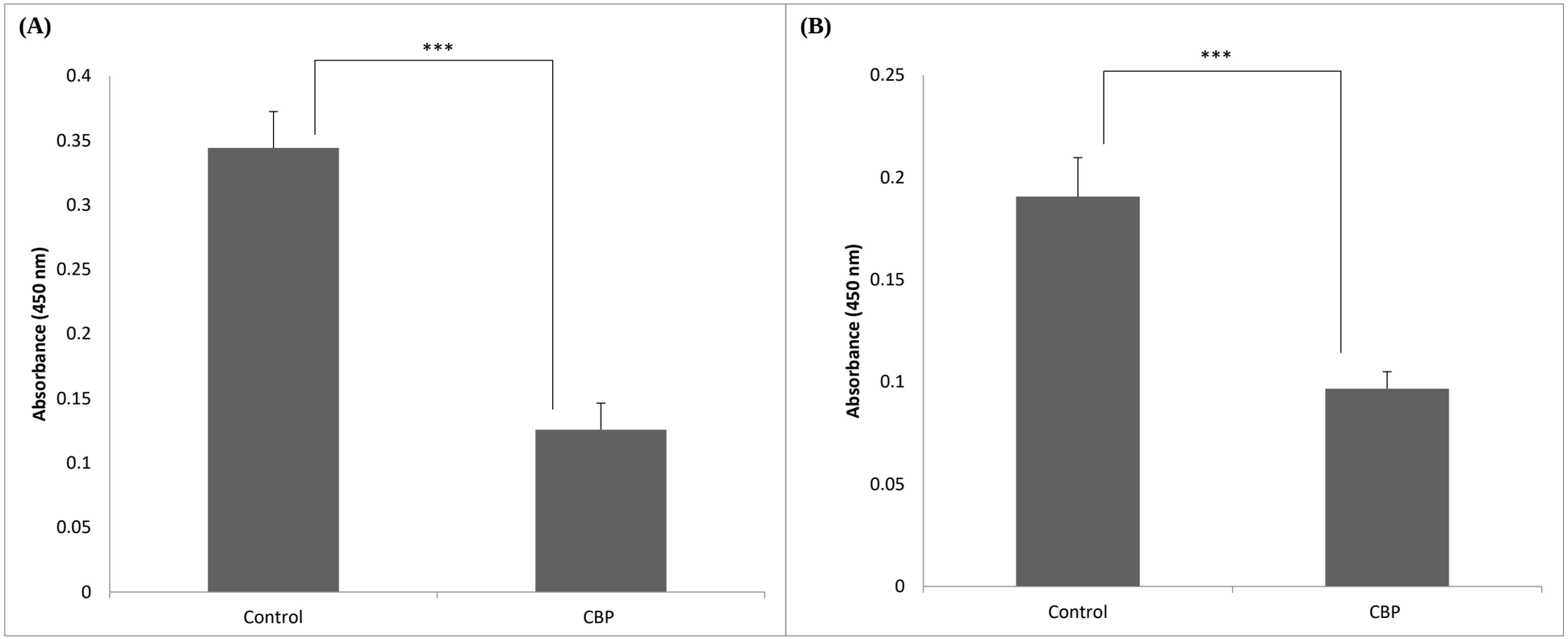


Figure 3

Effect of CBP (2 µg/well) on adhesion of *C. jejuni* strains 81-176 (A) and 11168H (B) in binding to collagen I, the bars labelled 'control' have respective *C. jejuni* strains ($2x10^7$ cfu/well) only, while that labelled CBP has a mixture of 2 µg CBP and $2x10^7$ cfu/well *C. jejuni*; the data represent three biological repeats each with three technical repeats (n=9).

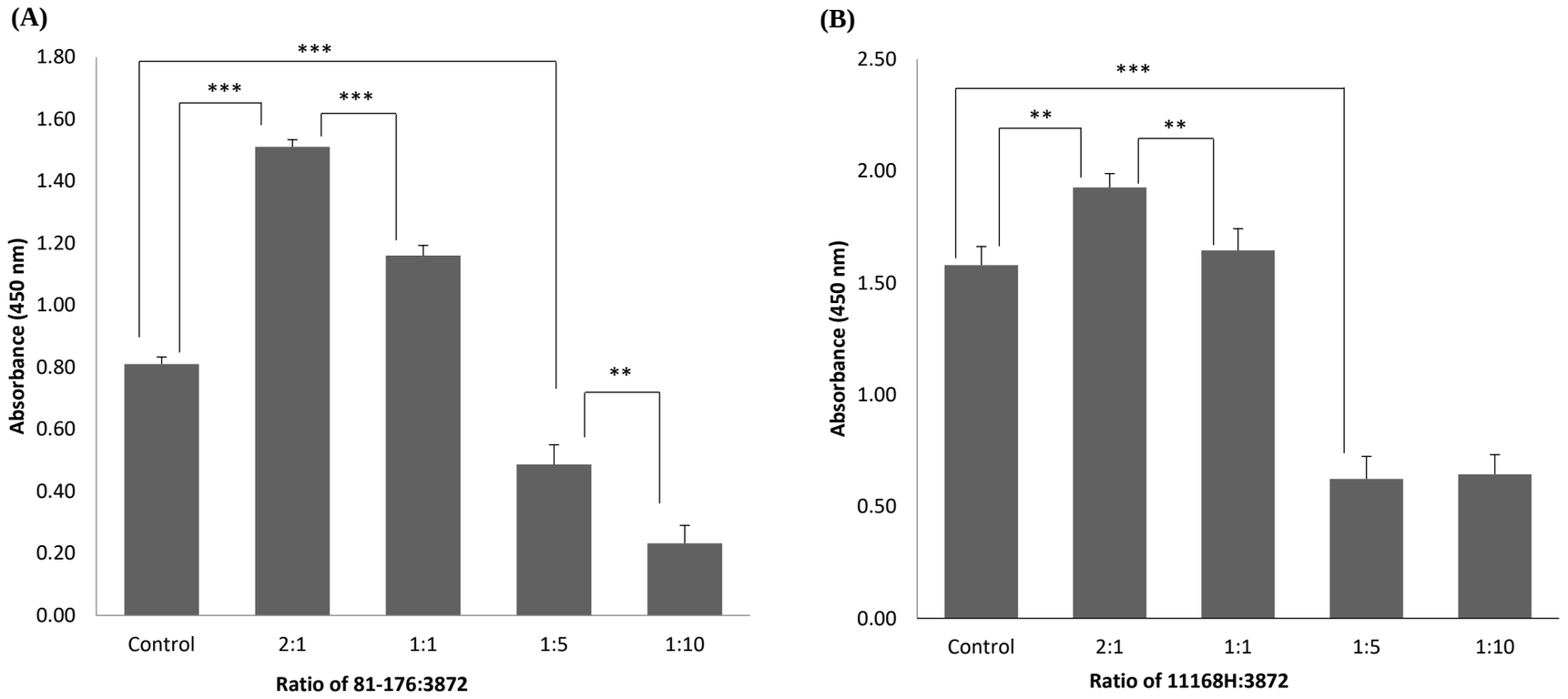


Figure 4

Competition between *L. fermentum* 3872 and *C. jejuni* strains 81-176 (A) and 11168H (B) for binding to collagen I detected using ELISA, the bars labelled 'control' have respective *C. jejuni* strains of amount $2x10^7$ cfu/well added only, the ratios indicated on the graph are based on cell to cell amounts added to each well; the data represent two biological repeats each with three technical repeats (n=6). Control (B) and 1:10 (B) represent data with three biological repeats each with three technical repeats each (n=9).

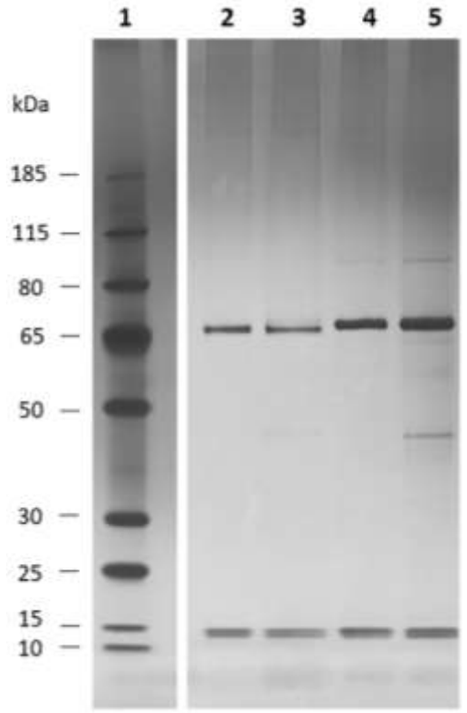
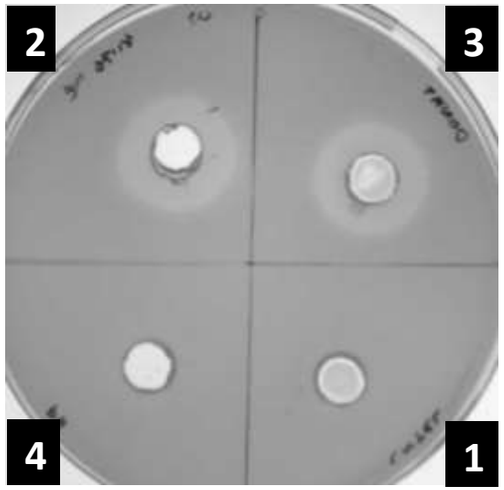


Figure 5

Silver staining of *C. jejuni* Co-IP eluate; 1, pre-stained ladder (Page ruler plus); 2, 11168H eluate after 1 hour incubation; 3, 11168H eluate after 3 hour incubation; 4, 81-176 eluate after 1 hour incubation; 5, 81-176 eluate after 3 hour incubation.

A) *C. jejuni* 81-176



B) *C. jejuni* 11168H

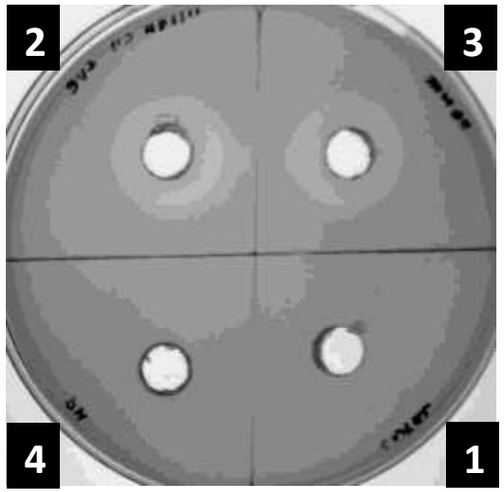


Figure 6

Inhibition of growth of *C. jejuni* strains 81-176 (A) and 11168H (B) in the presence of cell-free *L. fermentum* 3872 culture supernatant; 1, M.R.S broth (control); 2-4, *L. fermentum* 3872 cell-free supernatant; 2, untreated; 3, heat-treated; 4, pH adjusted. Three biological repeats were carried out.

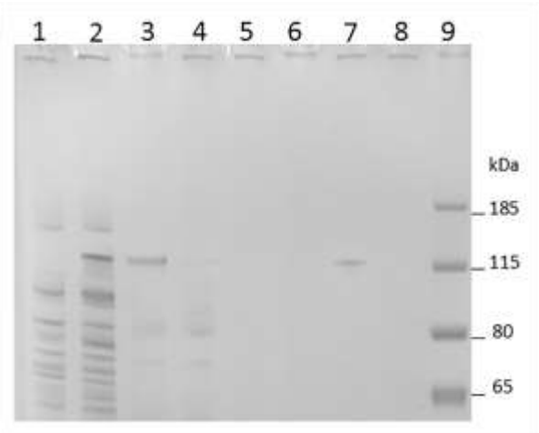


Figure S1

Coomassie staining of the recombinant CBP: 1, lysate before induction; 2, lysate after 3 hours induction; 3, clarified lysate; 4, flowthrough; 5, wash; 6, eluate 1; 7, eluate 2; 8, eluate 3; 9, pre-stained ladder (Page ruler plus).

1. WP_011812789.1 Mass: 59507 Score: 1866 Matches: 43(43) Sequences: 26(26) emPAI: 14.93
flagellin A [Campylobacter jejuni]
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Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
485	424.7267	847.4389	847.4399	-1.22	0	58	1.5e-06	1	U	K.SLDASLSR.L
515	426.7439	851.4733	851.4753	-2.30	0	41	8.4e-05	1		K.TTGVYAIK.E
975	466.2634	930.5123	930.5134	-1.22	0	28	0.0031	1		K.LVLTSADGR.G
1142	482.2588	962.5031	962.5033	-0.18	0	45	3.9e-05	1		K.ATIGATQSSK.I
1572	525.2685	1048.5224	1048.5223	0.14	0	52	6.3e-06	1		R.TMLQADINK.L + Oxidation (M)
2120	564.8218	1127.6290	1127.6298	-0.74	0	72	6.8e-08	1	U	R.INTNVAALNAK.A
2121	564.8218	1127.6291	1127.6298	-0.63	0	(59)	1.3e-06	1	U	R.INTNVAALNAK.A
2785	609.3086	1216.6026	1216.6048	-1.76	0	(32)	0.00087	1		K.ATQAAQDQQSLK.T
2786	609.3095	1216.6045	1216.6048	-0.25	0	73	6.4e-08	1		K.ATQAAQDQQSLK.T
4914	716.8145	1431.6145	1431.6154	-0.64	0	82	6.4e-09	1	U	R.DVDFASESANYSK.A
5067	482.9232	1445.7477	1445.7474	0.23	1	63	6.2e-07	1		K.TKATQAAQDQQSLK.T
5095	483.8988	1448.6745	1448.6743	0.11	1	41	7.9e-05	1		K.DTTGVQASKDENGK.L
5101	484.2265	1449.6577	1449.6583	-0.45	1	(24)	0.0045	1		K.DTTGVQASKDENGK.L + Deamidated (NQ)
5326	492.2544	1473.7415	1473.7423	-0.59	1	(68)	2.6e-07	1		K.DENGKLVLTSADGR.G
5334	492.5836	1474.7289	1474.7263	1.71	1	77	3e-08	1		K.DENGKLVLTSADGR.G + Deamidated (NQ)
6132	771.9282	1541.8418	1541.8413	0.29	0	110	1e-11	1		K.ITGDIGVGSGILANQK.E
6133	514.9549	1541.8429	1541.8413	1.00	0	(52)	6.9e-06	1		K.ITGDIGVGSGILANQK.E
7495	847.9238	1693.8330	1693.8370	-2.39	0	98	1.5e-10	1		K.TTAANTTDETAGVTTLK.G
7959	876.4444	1750.8742	1750.8737	0.29	0	88	1.6e-09	1	U	K.EGTTSQEFAINGVTIGK.I
7967	876.9330	1751.8514	1751.8578	-3.62	0	(75)	4e-08	1	U	K.EGTTSQEFAINGVTIGK.I + Deamidated (NQ)
8104	887.9067	1773.7988	1773.7992	-0.21	0	(93)	4.9e-10	1		K.GQISATNADAMGFNSYK.G
8207	597.6040	1789.7902	1789.7941	-2.19	0	(61)	7.3e-07	1		K.GQISATNADAMGFNSYK.G + Oxidation (M)

2. WP_011812788.1 Mass: 59696 Score: 1514 Matches: 35(35) Sequences: 21(21) emPAI: 7.67
flagellin B [Campylobacter jejuni]
Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
515	426.7439	851.4733	851.4753	-2.30	0	41	8.4e-05	1		K.TTGVYAIK.E
975	466.2634	930.5123	930.5134	-1.22	0	28	0.0031	1		K.LVLTSADGR.G
1142	482.2588	962.5031	962.5033	-0.18	0	45	3.9e-05	1		K.ATIGATQSSK.I
1572	525.2685	1048.5224	1048.5223	0.14	0	52	6.3e-06	1		R.TMLQADINK.L + Oxidation (M)
2785	609.3086	1216.6026	1216.6048	-1.76	0	(32)	0.00087	1		K.ATQAAQDQQSLK.T
2786	609.3095	1216.6045	1216.6048	-0.25	0	73	6.4e-08	1		K.ATQAAQDQQSLK.T
4788	708.8167	1415.6189	1415.6205	-1.14	0	43	4.9e-05	1	U	R.DVDFASESANYSK.Y
5067	482.9232	1445.7477	1445.7474	0.23	1	63	6.2e-07	1		K.TKATQAAQDQQSLK.T
5095	483.8988	1448.6745	1448.6743	0.11	1	41	7.9e-05	1		K.DTTGVQASKDENGK.L
5101	484.2265	1449.6577	1449.6583	-0.45	1	(24)	0.0045	1		K.DTTGVQASKDENGK.L + Deamidated (NQ)
5326	492.2544	1473.7415	1473.7423	-0.59	1	(68)	2.6e-07	1		K.DENGKLVLTSADGR.G
5334	492.5836	1474.7289	1474.7263	1.71	1	77	3e-08	1		K.DENGKLVLTSADGR.G + Deamidated (NQ)
6132	771.9282	1541.8418	1541.8413	0.29	0	110	1e-11	1		K.ITGDIGVGSGILANQK.E
6133	514.9549	1541.8429	1541.8413	1.00	0	(52)	6.9e-06	1		K.ITGDIGVGSGILANQK.E
7495	847.9238	1693.8330	1693.8370	-2.39	0	98	1.5e-10	1		K.TTAANTTDETAGVTTLK.G
8104	887.9067	1773.7988	1773.7992	-0.21	0	(93)	4.9e-10	1		K.GQISATNADAMGFNSYK.G
8207	597.6040	1789.7902	1789.7941	-2.19	0	(61)	7.3e-07	1		K.GQISATNADAMGFNSYK.G + Oxidation (M)
8208	895.9044	1789.7943	1789.7941	0.11	0	128	1.6e-13	1		K.GQISATNADAMGFNSYK.G + Oxidation (M)
8227	897.4177	1792.8209	1792.8261	-2.91	0	(47)	1.9e-05	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8228	598.6146	1792.8220	1792.8261	-2.28	0	(68)	1.5e-07	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8229	897.4184	1792.8222	1792.8261	-2.15	0	140	9.5e-15	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8247	897.9107	1793.8069	1793.8101	-1.81	0	(24)	0.0039	1		R.INSAADDASGMAIADSLR.S + Deamidated (NQ); Oxidation (M)
8592	921.4844	1840.9543	1840.9571	-1.51	0	87	3.4e-09	1		R.FETGAQSFTSGVVGLTIK.N

A) *C. jejuni* 81-176

1. YP_002344727.1 Mass: 58003 Score: 1108 Matches: 21(21) Sequences: 17(17) emPAI: 3.29
flagellin A [Campylobacter jejuni subsp. jejuni NCTC 11168 = ATCC 700819]
Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
483	424.7268	847.4351	847.4359	-1.01	0	60	1e-06	1	U	K.SLDASLSR.L
962	462.7427	923.4709	923.4713	-0.35	0	41	8.1e-05	1		R.ATFTVETR.G
1150	482.2590	962.5034	962.5033	0.13	0	36	0.00036	1		K.ATIGATQSSK.I
1719	539.2708	1076.5271	1076.5284	-1.26	0	51	8.1e-06	1		R.TMLQADINR.L + Oxidation (M)
2131	564.8219	1127.6292	1127.6298	-0.52	0	64	3.8e-07	1	U	R.INTNVAALNAK.A
2829	609.3094	1216.6042	1216.6048	-0.44	0	70	1.4e-07	1		K.ATQAAQDQQSLK.T
4035	676.3463	1350.6779	1350.6779	0.00	0	(29)	0.002	1	U	R.AGATSDTFAINGVK.I
4047	676.8371	1351.6596	1351.6620	-1.71	0	99	1.4e-10	1	U	R.AGATSDTFAINGVK.I + Deamidated (NQ)
4726	708.8174	1415.6203	1415.6205	-0.10	0	80	9.8e-09	1	U	R.DVDFAASESANYSK.A
5040	482.9234	1445.7463	1445.7474	0.61	1	32	0.00077	1		K.TKATQAAQDQQSLK.T
8233	598.6161	1792.8266	1792.8261	0.29	0	(45)	3.8e-05	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8234	897.4213	1792.8280	1792.8261	1.04	0	120	1.2e-12	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8268	898.4160	1794.8175	1794.8206	-1.76	0	95	3.5e-10	1	U	K.GQIDANIADAMGFGSANK.G + Oxidation (M)
9082	638.3021	1911.8945	1911.8884	-2.01	0	(60)	8.9e-07	1		R.LMEELDNIANTTSFNGK.Q + Oxidation (M)
9083	956.9497	1911.8849	1911.8884	-1.84	0	115	2.8e-12	1		R.LMEELDNIANTTSFNGK.Q + Oxidation (M)

2. YP_002344726.1 Mass: 59150 Score: 526 Matches: 11(11) Sequences: 9(9) emPAI: 1.07
flagellin B [Campylobacter jejuni subsp. jejuni NCTC 11168 = ATCC 700819]
Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
962	462.7427	923.4709	923.4713	-0.35	0	41	8.1e-05	1		R.ATFTVETR.G
1150	482.2590	962.5034	962.5033	0.13	0	36	0.00036	1		K.ATIGATQSSK.I
1719	539.2708	1076.5271	1076.5284	-1.26	0	51	8.1e-06	1		R.TMLQADINR.L + Oxidation (M)
2829	609.3094	1216.6042	1216.6048	-0.44	0	70	1.4e-07	1		K.ATQAAQDQQSLK.T
5040	482.9234	1445.7463	1445.7474	0.61	1	32	0.00077	1		K.TKATQAAQDQQSLK.T
7080	822.8682	1643.7219	1643.7250	-1.86	0	41	7.9e-05	1	U	R.FDANIADAMGFGSANK.G + Oxidation (M)
8233	598.6161	1792.8266	1792.8261	0.29	0	(45)	3.8e-05	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
8234	897.4213	1792.8280	1792.8261	1.04	0	120	1.2e-12	1		R.INSAADDASGMAIADSLR.S + Oxidation (M)
9082	638.3021	1911.8945	1911.8884	-2.01	0	(60)	8.9e-07	1		R.LMEELDNIANTTSFNGK.Q + Oxidation (M)
9083	956.9497	1911.8849	1911.8884	-1.84	0	115	2.8e-12	1		R.LMEELDNIANTTSFNGK.Q + Oxidation (M)
10475	743.3902	2227.0287	2227.0328	-1.85	1	20	0.01	1		K.IDGNIGGSAFIHAGMEESYSR.I + Oxidation (M)

B) *C. jejuni* 11168H

Figure S2

Screenshots of Mascot analysis of LC MS/MS output data; the diagram shows predominant hits for a 65 kDa collagen binding protein detected in *C. jejuni* strains 81-176 (A), 11168H (B).