Bacteriophage Receptor Binding Protein Based Assays for the Simultaneous Detection of Campylobacter jejuni and Campylobacter coli

Muhammad A. Javed1*, Somayyeh Poshtiban2, Denis Arutyunov1, Stephane Evoy2, Christine M. Szymanski1*

1 Alberta Glycomics Centre and Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, 2 Department of Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta, Canada

Abstract

Campylobacter jejuni and Campylobacter coli are the most common bacterial causes of foodborne gastroenteritis which is occasionally followed by a debilitating neuropathy known as Guillain-Barré syndrome. Rapid and specific detection of these pathogens is very important for effective control and quick treatment of infection. Most of the diagnostics available for these organisms are time consuming and require technical expertise with expensive instruments and reagents to perform. Bacteriophages bind to their host specifically through their receptor binding proteins (RBPs), which can be exploited for pathogen detection. We recently sequenced the genome of C. jejuni phage NCTC12673 and identified its putative host receptor binding protein, Gp047. In the current study, we localized the receptor binding domain to the C-terminal quarter of Gp047. CC-Gp047 could be produced recombinantly and was capable of agglutinating both C. jejuni and C. coli cells unlike the host range of the parent phage which is limited to a subset of C. jejuni isolates. The agglutination procedure could be performed within minutes on a glass slide at room temperature and was not hindered by the presence of buffers or nutrient media. This agglutination assay showed 100% specificity and the sensitivity was 95% for C. jejuni (n = 40) and 90% for C. coli (n = 19). CC-Gp047 was also expressed as a fusion with enhanced green fluorescent protein (EGFP). Chimeric EGFP-CC-Gp047 was able to specifically label C. jejuni and C. coli cells in mixed cultures allowing for the detection of these pathogens by fluorescent microscopy. This study describes a simple and rapid method for the detection of C. jejuni and C. coli using engineered phage RBPs and offers a promising new diagnostics platform for healthcare and surveillance laboratories.

Introduction

Campylobacter jejuni and Campylobacter coli are the major foodborne pathogens that cause enteritis and diarrhea [1]. Campylobacter infection is generally self-limiting, however, it can be followed by post-infectious sequelae including the autoimmune neurological disorder, Guillain-Barré syndrome [2].

Poultry and swine are the natural hosts for C. jejuni and C. coli and are thus the main sources of campylobacteriosis through the ingestion of contaminated food [1]. Rapid detection of these pathogens is needed to avoid the spread through the food-chain and for quick treatment and effective control. Generally, campylobacters are isolated from any sample using filtration, culture enrichment and growth on selective media followed by identification using microscopy and biochemical assays. Though traditional culture based identification of bacterial isolates remains a gold standard, these conventional methods are laborious and time consuming. Over the last decade, many efforts have been made to develop tools for the rapid detection of Campylobacter species, especially C. jejuni and C. coli. After the publication of the first C. jejuni genome sequence in 2000 [3], most of the reported

rapid detection methods are based on the polymerase chain reaction (PCR) including multiplex and real-time PCR [4,5,6] and require DNA isolation, removal of PCR inhibitors from the samples, expensive instruments and reagents, as well as significant expertise to run these tests. Serological typing of campylobacters is also a widely used technique however, cost of antibody production and their shorter shelf life are limitations for this method. Thus, there is a need for rapid, specific, cost-effective and simple methods for the simultaneous detection of the two major foodborne pathogens, C. jejuni and C. coli. Several new techniques have recently been described for the detection of these bacteria [4,5,6,7,8,9,10,11], and any novel technique is expected to be superior in at least one major factor such as the specificity, rapidity, cost-effectiveness and/or ease of use.

Bacteriophages (or phages) have inherent host specificity and this specificity makes them excellent probes for identifying their target bacteria [12]. Phage typing schemes have been developed for many pathogens [13,14,15] including Campylobacter species [16,17,18]. Unfortunately, these schemes demand the laborious and time consuming preparation of bacterial cultures and may be limited by the problems associated with the production and
storage of these phages. We have also shown that phages immobilized on solid surfaces can be utilized for the detection of bacterial pathogens without the necessity for traditional culturing [19,20,21]. However, like other detection methods, immobilized phage-based assays have their shortcomings. For example, desiccation of surface-immobilized phages severely impairs their binding efficiency, and over exposure of the bacterial cells to the phage particles results in the lysis and the eventual destruction of the captured bacteria being detected. These drawbacks of the immobilized phage based assays can be avoided by using phage receptor binding proteins (RBPs) instead of the whole phage particles [12,22].

We recently sequenced the genome of the lytic C. jejuni phage NCTC12673 and identified a putative RBP, Gp047; previously annotated as Gp48 [23]. Gp047 does not share sequence homology with any characterized phage RBPs, but homologues are found in all sequenced campylobacter phages [24,25,26,27,28]. We have also shown that antibodies against Gp047 cross-react with native Salmonella enterica serovar Typhimurium phage P22 tail spike protein (TSP), but not denatured TSP, suggesting that these proteins have structural similarity [23]. In addition, Gp047 forms sodium dodecyl sulfate resistant oligomers [23], similar to the TSPs of Shigella flexneri and Shigella flexneri phage SF6 [29,30], and can specifically capture C. jejuni NCTC11168 cells when immobilized onto solid surfaces [11]. This ability of immobilized Gp047 to capture C. jejuni can also be exploited for the preconcentration of campylobacter cells before using more traditional methods for analyzing samples suspected to be contaminated with this organism.

Since Gp047 is an oligomer and is the largest protein encoded in the genome of NCTC12673 (152 kDa), its large size poses a challenge for expressing an active form of the protein in high yields. Here, we report the localization of the binding domain in Gp047 that allowed us to generate a shorter derivative that is one-quarter the size of the original protein, yet is capable of detecting Gp047 that allowed us to generate a shorter derivative that is one-quarter the size of the original protein, yet is capable of detecting Gp047 including N-Gp047, NC-Gp047, 3C-Gp047 and 4C-Gp047 (residues 1204–1365) derivatives were immobilized onto solid surfaces. We also report a fluorescent microscopy based method for the detection of C. jejuni and C. coli in mixed cultures. To our knowledge, this is the first report of a phage RBP-based assay for the simultaneous detection of these Campylobacter species.

**Results**

**Localization of the Gp047 Binding Domain**

The purified GST-Gp047 wildtype, N-Gp047 (residues 1–684), C-Gp047 (residues 681–1365), NC-Gp047 (residues 681–1041), CC-Gp047 (residues 1040–1365), 3C-Gp047 (residues 1040–1204) and 4C-Gp047 (residues 1204–1365) derivatives were immobilized onto solid surfaces and were tested for their ability to capture C. jejuni NCTC11168 cells. The NCTC11168 cells were captured by Gp047 wildtype, C-Gp047 and CC-Gp047, but not by N-Gp047 or NC-Gp047 indicating that the host binding domains were localized in the C-terminal quarter of Gp047 (Fig. 1). Further truncation of CC-Gp047 into 3C-Gp047 and 4C-Gp047 resulted in the complete loss of binding (Fig. 1, 3C-Gp047 not shown).

**Confirmation of Host Binding Specificity**

Host binding specificity was also tested through immunolabeling of the bacterial cells with CC-Gp047 followed by detection with anti-Gp047 antibodies and Alexa Fluor®546 conjugated secondary antibodies. Fluorescent microscopy of the samples showed that CC-Gp047 binds specifically to C. jejuni NCTC11168, but not to S. Typhimurium which was used as a negative control (Fig. 2). The DNA intercalating dye, DAPI, stained both C. jejuni as well as S. Typhimurium cells.

**Agglutination Assay**

An agglutination assay combining C. jejuni NCTC11168 cells with CC-Gp047 formed aggregates within one minute (Fig. 3). It has previously been demonstrated that C. jejuni is also capable of autoagglutinating [31]. To rule out the possibility that bacteria were autoagglutinating, we always included a control suspension of the respective bacterial strain in PBS without CC-Gp047. The results showed that agglutination was caused by CC-Gp047 and was not due to autoagglutination since control suspensions lacking CC-Gp047 did not show agglutination. Other derivatives of Gp047 including N-Gp047, NC-Gp047, 3C-Gp047 and 4C-Gp047 did not cause agglutination of C. jejuni NCTC11168.

Next, the bacterial cells were suspended in different growth media (MH, BHI, LB, and NCZYM) as well as in different buffers (phosphate buffer pH 7.4, HEPES buffer pH 7.4, normal saline, Tris-HCl buffer pH 7.5 and 5% BSA in PBS) to determine if the agglutination assay can be performed in different salts and complex media. The assay showed the same results in all tests. To test if bacterial cell growth phase or viability has an effect on the agglutination assay, we tested C. jejuni NCTC11168 cells collected after 18 and 62 hours growth containing cells in different growth states with the majority in the viable but non-culturable state in the 62 hour culture. Both preparations showed agglutination with CC-Gp047. In addition, formalin killed C. jejuni NCTC 11168 cells showed agglutination with CC-Gp047. These results suggest that the growth state has no influence on the recognition of the bacterial cells by this protein and the agglutination assay does not differentiate between live or dead cells.

We also tested the stability and shelf-life of the protein by testing its ability to agglutinate C. jejuni NCTC11168 at different time points after purification. CC-Gp047 was able to consistently agglutinate the bacterial cells up to the latest tested time point of 18 months after being stored at 4°C (data not shown).

**Specificity and Sensitivity of the Assay**

To check the host range of CC-Gp047, we tested several campylobacter strains isolated from a variety of sources including symptomatic and asymptomatic human patients, animal and animal products, environment and laboratory strains. In total, 40 strains of C. jejuni, 19 strains of C. coli, and representative strains of other Campylobacter species including C. fetus subsp. fetus, C. fetus subsp. vulneralis, C. lari, C. upsaliensis and C. concisus were tested. In addition, we tested two strains of the related human pathogen Helicobacter pylori, two laboratory strains of E. coli, and S. Typhimurium. In total 38 out of 40 (95%) isolates of C. jejuni showed agglutination when mixed with CC-Gp047 and unexpectedly 17 out of 19 (90%) isolates of C. coli were also agglutinated in the presence of CC-Gp047 (Table 1). The assay showed 100% specificity for C. jejuni and C. coli as none of the other Campylobacter species, H. pylori, E. coli or S. Typhimurium showed agglutination with CC-Gp047 (Table 1). This confirmed that the assay can be used for the specific and simultaneous detection of C. jejuni and C. coli. Interestingly, CC-Gp047 showed a much broader host range in the agglutination assays compared to the lytic host range of phage NCTC 12673 from where Gp047 was derived from.
Direct Detection of C. jejuni and C. coli using EGFP-fused CC-Gp047

For the detection of C. jejuni and C. coli in mixed cultures, we generated CC-Gp047 fused with the fluorescent reporter protein, EGFP. Then C. jejuni NCTC11168, C. coli RM2228 and E. coli DH5α cell suspensions were individually incubated with chimeric EGFP_CC-Gp047, washed and observed under a fluorescent microscope. The C. jejuni and C. coli cells were detected as green fluorescing cells (Fig. 4: A, C) indicating the binding of EGFP_CC-Gp047 while the E. coli DH5α cells (Fig. 4: E) did not show fluorescence, as expected. This confirmed that CC-Gp047 maintains its binding activity and specificity when expressed as a chimeric EGFP_CC-Gp047 protein. We then prepared mixed cultures of C. jejuni NCTC11168 and E. coli DH5α by mixing the respective cell suspensions at a ratio of 1:25 (cell numbers) and tested if EGFP_CC-Gp047 can be used to specifically detect C. jejuni from a mixed culture. Only C. jejuni cells were specifically labelled (Fig. 4: G) in the mixed culture. We also repeated the detection assay using whole cell lysates of IPTG induced E. coli C43 cells expressing EGFP_CC-Gp047. Fluorescent microscopy of the bacterial cells exposed to the EGFP_CC-Gp047 containing lysate still showed the effective labelling of C. jejuni NCTC11168 and C. coli RM2228, but not E. coli DH5α cells (not shown) suggesting that purification of the EGFP_CC-Gp047 is not necessary for the detection assay. This further increases the ease of EGFP_CC-Gp047 use for the detection of C. jejuni and C. coli.

Discussion

C. jejuni and C. coli are the major causative agents of campylobacteriosis and are responsible for more than 95% of human cases. We exploited the specificity of Gp047, the putative RBP of C. jejuni phage NCTC12673, to develop assays for the rapid detection of C. jejuni and C. coli. Gp047 does not exhibit any homology with other characterized phage RBPs using the basic alignment search tool (blast.ncbi.nlm.nih.gov/) or Pfam searches (http://pfam.sanger.ac.uk/). Thus, it was difficult to predict the location of the binding domains in this protein. Interestingly, the C-terminal part of the Gp047 is conserved in all the campylobacter phages where genome sequence data is available [28] and this is consistent with the results from this study which revealed that the host binding domain was also localized in the C-terminal part of this protein. The host binding domains of other phage RBPs have also been reported to be localized in the C-terminal [32,33,34] or the central portion of the protein [35] while the N-terminus is generally involved in the attachment of the tail receptor binding elements (either long fibers or short tail spikes) to the phage particle body [32,33,34]. The role of the N-terminus of Gp047 is yet not known, however, like most other tailed-phage RBPs, the C-terminus of this protein was found to be responsible for bacterial host recognition.

Receptor binding proteins of many phages including the TSPs of the well-characterized S. Typhimurium phage P22 and S. flexneri phage SF6 form trimers [29,30]. It was shown that Gp047 also forms oligomers [23]. Similar to the full-length Gp047, CC-Gp047 also formed trimers and higher order SDS-resistant oligomers (data not shown). This observation explains the ability of CC-Gp047 to agglutinate C. jejuni cells.

Interestingly, Gp047 was found to agglutinate a broader spectrum of bacterial strains compared to the lytic range of phage NCTC12673. A broader host spectrum was also observed for other phage-derived proteins compared to their host phage. For example, the recombinant cell binding domain (CBDP40) derived from listeria phage P40 showed broader binding activity than phage P40 [36]. Similarly, the recombinant phage lysis (LysK) derived from staphylococcus phage K had broader lytic activity compared to whole phage K [37].

Our RBP-based agglutination assay is very rapid and requires only a glass slide so the method can be used to complement any of the traditional methods. Indeed, this agglutination assay showed 100% specificity for C. jejuni and C. coli and 95% and 90% sensitivity for C. jejuni and C. coli, respectively which is better or comparable to other Campylobacter species detection methods. Bessêde et al. compared conventional campylobacter culture

Figure 1. Scanning electron microscopy images of Campylobacter jejuni cells captured by immobilized Gp047 and its derivatives. GST-fused Gp047 (A), truncated N-Gp047 (B), C-Gp047 (C), NC-Gp047 (D), CC-Gp047 (E), and 4C-Gp047 (F) were immobilized onto gold-coated surfaces and incubated with C. jejuni NCTC11168 cells. Scale bar represents 10 μm. doi:10.1371/journal.pone.0069770.g001
Figure 2. Immunofluorescence microscopy images of *Campylobacter jejuni* cells after incubation with CC-Gp047. CC-Gp047 showed binding to *C. jejuni* NCTC11168 (A) but not to *S. enterica* serovar Typhimurium (C). DAPI stained *C. jejuni* NCTC11168 (B) and *S. Typhimurium* (D). Scale bar represents 10 μm.
doi:10.1371/journal.pone.0069770.g002

Figure 3. Agglutination of *Campylobacter jejuni* NCTC11168 cells with CC-Gp047. *C. jejuni* NCTC11168 cells were agglutinated with CC-Gp047 but not with PBS alone.
doi:10.1371/journal.pone.0069770.g003
enrich bacterial cells. We have also developed an assay to separate and complex growth media do not influence the binding of Gp047 to demonstrating that different salts, salt concentrations as well as assays providing greater flexibility for the users. The results commonly used growth media and buffers for the agglutination storage. And, bacteria can be resuspended in any of the most indicating that the protein remains stable during prolonged labor and/or time consumption as well as the need for expensive sensitivity and specificity while lacking the drawbacks associated Gp047 RBP-based detection method offers comparable levels of used a fluorescent hybridization technique to detect Campylobacter species and observed a sensitivity of 90% and 97% for C. jejuni and C. coli from a sample containing a mixture of bacterial species, CC-Gp047 was expressed as a chimeric protein fused with a reporter fluorescent protein. The EGFP_CC-Gp047 chimera recognized both C. jejuni and C. coli cells and was able to specifically detect these cells even when they were in low numbers or in a mixed culture. Like other fluorescence based assays, the presence of fluorescence quenchers or autofluorophores in the sample may affect the efficiency of this assay. If required, these potential inhibitors can be removed by centrifugation or magnetic bead enrichment techniques.

Therefore we describe two rapid, simple and specific assays that provide proof-of-principle that a short derivative of the campylobacter phage receptor protein, CC-Gp047, can be used for the simultaneous detection of the major pathogenic Campylobacter species (C. jejuni and C. coli). These assays are not technically demanding and do not need special instrumentation, with the exception of a fluorescent microscope for the EGFP assays. Furthermore, since bacteriophage abundance is estimated at 10^{31} particles worldwide, they represent a limitless resource for the methods to two molecular biology methods: an in-house real-time PCR and a multiplex PCR named Seeplex Diarrhea ACE Detection, and three immunoenzymatic methods: Premier Campy, RidaScreen Campylobacter, and ImmunoCard Stat-Campy. The authors found that the specificity of all these methods was in the range of 95 to 100% while sensitivity was 80% for the in-house PCR, 88% for the multiplex PCR, 95% for the Premier Campy and 91% for the immunoCard method [38]. Poppert et al. 2005 [45], et al. 2007 [41], et al. 2005 [44], et al. submitted). Strains highlighted in bold were also tested for lysis by Campylobacter phage NCTC12673. The asterisks indicate strains unable to be lysed by the phage. doi:10.1371/journal.pone.0069770.t001

| Organism Strains | Sources/disease | Agglutination |
|------------------|----------------|--------------|
| C. jejuni Cj60, B1–176, NCTC12517, HS3, NCTC11168, NCTC11168H, RM1244 | Human/Enteritis | Positive |
| Cj31467, Cj31481, Cj31485, Cj32787, Cj33084, Cj33106 | Human/Asymptomatic | Positive |
| RM1221, RM1814, RM1216, RM1235, RM1850, RM2668, RM1462, Cj120, Cj150, Cj160, Cj178, Cj198, Cj206, Cj207, Cj220, Cj230, Cj1919, Cj1974, Cj12567, NCTC12658, NCTC12659, NCTC12660, NCTC12661, NCTC12663, NCTC12664, NCTC12665 | Animal/Animal products | Positive |
| Cj212, Cj11848 | Animal/Animal products | Negative |
| C. coli ROH1, ROH2, Cc167, Cc345, Cc423, Cc456 | Frozen poultry | Positive |
| Cc11, Cc145, Cc253, Cc255, Cc525, Cc700, NCTC12532, RM1515, RM2228 | Animal/animal products | Positive |
| RM1891 | Chicken farm | Positive |
| RM1900 | Swine farm | Positive |
| VC167, Cc463 | Human, Animal | Negative |
| C. lari RM2100 | Human | Negative |
| C. fetus fetus NCTC10482 | Sheep/Abortion | Negative |
| C. fetus venerealis NCTC10354 | Cow/Venereal disease | Negative |
| C. concisus NCTC11485 | Human/Periodontitis | Negative |
| C. upsaliensis RM3195 | Human/Guillain-Barré syndrome | Negative |
| H. pylori 399, G27 | Human | Negative |
| E. coli DH5a, BL21 | Invitrogen | Negative |
| S. enterica serovar Typhimurium ATCC 19585 | ATCC 19585 | Negative |

*McNally, D. et al. 2007 [41],
*Black, R. E. et al. 1988 [42],
Parkhill, J. et al. 2000 [3],
*Miller, W. G. et al. 2005 [43],
*Champion, O. L. et al. 2005 [44],
*Fouts, D. E. et al. 2005 [45],
*Mandrell, R.E. et al. 2005 [46],
*Alm, R. A. et al. 1999 [47],
*Covacci, A. et al. 1993 [48].

List of bacterial strains used and results of agglutination assay.
Figure 4. Fluorescent microscopy images of mixtures of *Escherichia coli*, *Campylobacter jejuni* and/or *Campylobacter coli* cells after incubation with EGFP_CC-Gp047. Bacterial cell suspensions were incubated with EGFP_CC-Gp047 and imaged by both fluorescence microscopy (A, C, E, G) and bright field microscopy (B, D, F, H). EGFP_CC-Gp047 bound specifically to *C. jejuni* NCTC11168 (A, B), *C. coli* RM2228 (C, D) but not to *E. coli* DH5α (E, F). EGFP_CC-Gp047 also showed specific binding to *C. jejuni* NCTC11168 in a mixed suspension of *C. jejuni* NCTC11168 and *E. coli* DH5α at a ratio of 1:25 (G, H). Scale bar represents 10 μm.

doi:10.1371/journal.pone.0069770.g004
development of novel diagnostic platforms against any bacterial host.

**Materials and Methods**

**Bacterial Strains and Growth Conditions**

_C. jejuni_ and _C. coli_ strains were routinely grown on Mueller Hinton (MH) agar (Difco) at 37°C under microaerobic conditions (85% N₂, 10% CO₂, and 5% O₂). Other _Campylobacter_ species and _H. pylori_ strains were grown on MH agar supplemented with horse blood (5%, v/v). _S. enterica_ serovar Typhimurium was grown on MH agar under aerobic conditions. _Escherichia coli_ strains were grown on Luria Bertani (LB) agar supplemented with ampicillin, kanamycin or chloramphenicol; selected on LB agar supplemented with ampicillin. The in-frame bacterial strains used in this study are given in Table 1.

**Localization of Host Binding Domains in Gp047**

To localize the host binding domains, truncated versions of Gp047 were expressed as GST-fusions using the pGEX 6P-2 vector (GE Healthcare) and _E. coli_ BL21 cells (Invitrogen) unless otherwise mentioned. The lists of plasmids and primers used in this study are given in Table 2 and Table 3, respectively. Initially _gp047_ DNA fragments encoding for the **N**- (residues 1–684) and **C**- terminal (residues 681–1365) parts of Gp047 were amplified from pGEX_ _gp047_ [23] using primer pairs CS710/CS711 and CS712/CS713, respectively. Each amplicon was digested with _EcoRI_ and _XhoI_ and ligated with the _EcoRI_/_XhoI_ double digested pGEX 6P-2 vector. The resulting vectors pGEX_ntgp047 and pGEX_cctgp047 were transformed into _E. coli_ DH5α and then BL21; colonies were selected on LB agar supplemented with ampicillin. The in-frame cloning of the DNA fragments was confirmed by restriction analysis and nucleotide sequencing performed at the Molecular Biology Service Unit, Biological Sciences Department, University of Alberta.

The C-terminal half of Gp047 was further truncated into NC-Gp047 (residues 681–1041) and CC-Gp047 (residues 1040–1365); their encoding DNA fragments were amplified using primer pairs CS710/CS711 and CS712/CS713, respectively. The amplicons encoding for NC-Gp047 and CC-Gp047 were double digested with _EcoRI_ and _XhoI_ and cloned into pGEX 6P-2 as described above. The resulting vectors pGEX_ncgp047 and pGEX_ccgp047 were confirmed by restriction analysis and nucleotide sequencing.

Further truncations of CC-Gp047 were generated creating 3C-Gp047 (residues 1040–1204) and 4C-Gp047 (residues 1204–1365); DNA fragments encoding for 3C-Gp047 and 4C-Gp047 were amplified from pGEX_ccgp047 using primer pairs CS710/CS725 and CS726/CS713, respectively. PCR products were digested with _EcoRI_ and _XhoI_ and then cloned into pGEX 6P-2 as described above. The resulting vectors pGEX_3ccgp047 and pGEX_4ccgp047 were analysed by restriction analysis and nucleotide sequencing.

### Table 2. List of plasmids used in this study.

| Plasmid  | Description                               | Source or reference |
|----------|-------------------------------------------|---------------------|
| pGEX 6P-2 | Glutathione-S-transferase (GST) fused protein expression vector, ampicillin resistance marker, tac promoter | GE Healthcare       |
| pGEX_gp047 | _gp047_ in frame cloned into multiple cloning site of pGEX 6P-2 for expression of GST-fused Gp047 | [23]               |
| pGEX_ntgp047 | Expression construct of GST-fused N-Gp047 in pGEX 6P-2 | This study         |
| pGEX_ccgp047 | Expression construct of GST-fused C-Gp047 in pGEX 6P-2 | This study         |
| pGEX_phgp047 | Expression construct of GST-fused NC-Gp047 in pGEX 6P-2 | This study         |
| pET28b_ccgp047 | Expression construct of 6His-fused CC-Gp047 in pET28a | This study         |
| pET28a_6ccgp047 | Expression construct of 6His-fused CC-Gp047 in pET28a | This study         |
| pET28a | Protein expression vector, 6His tag, kanamycin resistance marker, T7 promoter | Invitrogen         |

### Table 3. List of primers used in this study.

| Primer   | Nucleotide sequence (5’→3’) |
|----------|-----------------------------|
| CS710    | GCCCCTGCGATCCCGAGGAATCC     |
| CS711    | *AAGCTCGAGTTGACGATGTACAGCTCGTCCATGCC |
| CS712    | *AAGGAATCCCGCACTTACCTCTTGAAATAACGAGAATCC |
| CS713    | CAGATCTGACGATCTGACGATTC    |
| CS716    | *AAGCTCGAGTTGACGATGTACAGCTCGTCCATGCC |
| CS717    | *AAGCTCGAGTTGACGATGTACAGCTCGTCCATGCC |
| CS725    | *AAGCTCGAGTTGACGATGTACAGCTCGTCCATGCC |
| CS726    | *AAGCTCGAGTTGACGATGTACAGCTCGTCCATGCC |
| CS1005   | *ATATTAGGTCATGTTGACGAGCGAGG |
| CS1006   | *ATATTAGGTCATGTTGACGAGCGAGG |

*Additional nucleotides and BamHI (gatc), EcoRI (gaatc), Ndel (catatg), or XhoI (ctcgcag) restriction site at 5’ end of the primers are underlined, where applicable.

doi:10.1371/journal.pone.0069770.t003
The egfp was amplified from pBAD/HisB_egfp (a gift from Robert E. Campbell, Chemistry Department, University of Alberta) with primers CS1005 and CS1006. The PCR products were digested with NdeI and BamHI and cloned into NdeI and BamHI digested pET28_ccgp047. The resulting plasmid pET28_egfp-ccgp047 was transformed into E. coli C43 cells and colonies were selected on LB agar supplemented with kanamycin. The in-frame cloning was confirmed by nucleotide sequencing of the constructs.

**Protein Expression and Purification**

The full GST-Gp047 protein and GST-fused Gp047 derivatives were expressed in E. coli BL21 cells and the proteins were purified as described previously [23].

The EGFP-fused CC-Gp047 was expressed in E. coli C43 cells. The bacterial cells harbouring pET28_ccgp047 or pET28_egfp-ccgp047 were grown at 30°C in 2xYE broth while shaking at 200 rpm. When the culture reached an OD600 of 0.3, the cells were induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG) and shaken for 6 hours. The culture was then stored overnight at 4°C to allow complete maturation of the EGFP chromophore. The cells were harvested by centrifugation at 10,000×g for 15 minutes, resuspended in buffer A (20 mM sodium phosphate, 500 mM NaCl, 30 mM imidazole, pH 7.4) supplemented with the protease inhibitor cocktail (Roche) and were lysed by using a cell disruptor (Constant Systems, UK). The lysate was centrifuged at 17400×g for 30 minutes followed by filtration through 0.2 μm syringe filters (Millipore). The protein was purified by immobilized metal ion affinity chromatography using a nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) column according to the manufacturer’s protocol. The captured protein was eluted from the Ni-NTA column using buffer B (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4) and dialyzed against 4 L of phosphate buffered saline (pH 7.5), with two buffer changes. The protein concentration was determined by measuring sample absorbance at 280 and 260 nm. The protein purity was determined by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie blue staining.

**Bacterial Capture by Immobilized GST-Gp047 and Derivatives**

The ability of Gp047 and its derivatives to capture C. jejuni was assayed as described previously [11]. Briefly, the gold substrates were washed sequentially in acetone, isopropyl alcohol, ethanol, and water for 5 min each to clean the surface prior to functionalization. The clean gold chips were incubated in a 2 mg/ml solution of glutathione in PBS for 30 min at room temperature. The substrates were washed, fixed, dried, and imaged using a scanning electron microscope (LEO 1430). The bacterial binding density was estimated from the images by using the cell counter plugin of ImageJ software (National Institutes of Health).

**Immunostaining of CC-Gp047 Bound to Bacterial Cells**

Bacterial cells that were grown overnight were resuspended in 50 mM Tris-HCl buffer (pH 7.5) and added to a clean glass slide. After air drying, the bacteria were fixed with 5% glutaraldehyde for 5 minutes and then blocked with blocking buffer (5% BSA in 50 mM Tris-HCl buffer, pH 7.5). The slide was put into purified GST-fused CC-Gp047 (10 μg/ml final concentration) solution prepared in blocking buffer and incubated for one hour followed by washing 3x with Tris-HCl buffer. The slide was then put into rabbit anti-Gp047 antibody (1:1000 dilution) solution [23] and incubated for one hour and then washed 3x with Tris-HCl buffer. The slide was then exposed to Alexa Fluor® 546 conjugated goat anti-rabbit IgG antibodies (Invitrogen) 1:500 diluted in blocking buffer for one hour, followed by washing 3x with Tris-HCl buffer and air drying with minimal exposure to light. The slide was then observed under a Leica DMRXA fluorescent microscope.

**Agglutination Assay**

Bacterial overnight growth was harvested from agar plates and resuspended in PBS to an OD600 ~1.5. A 50 μl aliquot of the cell suspension was mixed with 1 μl of CC-Gp047 (0.4 mg/ml) on a glass slide and swirled. Agglutination began to appear in the first minute, but the slide was swirled for up to 5 minutes in negative tests. The agglutination assay was also done using other derivatives of Gp047 including NC-Gp047, 3C-Gp047 and 4C-Gp047. Specificity or the true negative rate that measures the proportion of negatives which are correctly identified, and sensitivity or the true positive rate that measures the proportion of actual positive samples which are correctly identified were calculated using the formula, specificity = d/(b+d)×100, and sensitivity = a/(a+c)×100 where a: true positive; b: false positive; c: false negative and d: true negative.

The agglutination assay was performed with cells suspended in different growth media including MH, brain heart infusions, LB, and NZCYM broth as well as in phosphate buffer pH 7.4, HEPES buffer pH 7.4, saline (0.9% w/v sodium chloride in distilled water), Tris-HCl buffer pH 7.5, and 5% BSA in PBS.

The effect of bacterial cell viability on the agglutination assay was tested by using formalin killed cells. C. jejuni NCTC 11165 cells were suspended in PBS containing 2.5% formalin for 15 minutes, then cells were washed with PBS and finally resuspended in PBS.

**Phage Lytic Assay**

The phage lytic assay was performed as previously described [40] with minor modifications. Briefly, overnight bacterial growth was resuspended in brain heart infusions broth supplemented with 1 mM CaCl2 and 10 mM MgSO4 and incubated for 4 hours. Then the OD600 of the culture was adjusted to 0.2 and 50 μl of the suspension was mixed with 5 ml NZCYM (0.6% w/v sodium chloride in distilled water), Tris-HCl buffer pH 7.5, and 5% BSA in PBS. After solidification, the plates were spotted with 10 μl of phage suspensions. The spots were allowed to dry at room temperature before incubation for 24 h at 37°C under microaerobic conditions.

**Detection of Bacteria using Chimeric EGFP_CC-Gp047**

Bacterial growth was harvested from agar plates and the OD600 was adjusted to 0.3 in 50 mM Tris-HCl buffer (pH 7.5); 5 μl purified EGFP-Gp047 (1.0 mg/ml) was mixed with 200 μl of the cell suspension and incubated for 20 minutes at room temperature.
on a shaker. The cells were washed three times with 50 mM Tris-HCl buffer by centrifugation at 5000×g using a bench-top centrifuge for 2 minutes to remove unbound protein and finally resuspended in 100 μl buffer. The cells were then observed under a Leica DMRXA fluorescent microscope.

In addition to purified EGFP_CG-Gp047, the bacterial cells were also stained using whole cell lysates of IPTG induced E. coli C43 cells expressing EGFP_CC-Gp047. For the preparation of the cell lysate, E. coli C43 cells with pET28_egfp-cg-gp047 were grown in 2xYT broth and were induced by IPTG as described above. The induced cells were resuspended in 50 mM Tris-HCl buffer containing a protease inhibitor cocktail (Roche) and were sonicated. After centrifugation, the lysate was filter sterilized through a 0.2 μm syringe-driven filter and stored at 4°C overnight to allow complete maturation of the EGFP chromophore. Then 7 μl of the lysate (protein concentration 1.6 mg/ml) was mixed with 200 μl bacterial cell suspension and incubated for 20 minutes at room temperature on a shaker, washed 3X and then resuspended in 100 μl Tris-HCl buffer.

Acknowledgments

The authors are thankful to Dr. Robert Campbell for the gift of egf, Dr. Mario Feldman for providing the H. pylori strains, Dr. Amit Singh for help with the binding assays and Arlene Oatway (Advanced Microscopy Facility, Department of Biological Sciences, University of Alberta) for help with fluorescent microscopy.

Author Contributions

Conceived and designed the experiments: MAJ. Performed the experiments: MAJ SP. Analyzed the data: MAJ SP DA SE CMS. Wrote the paper: MAJ CMS DA.
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