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# Screening of Genes Expressed *In Vivo* During Interaction Between Chicken and *Campylobacter jejuni*

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Chicken are considered as the most important source of human infection by *Campylobacter jejuni*, which primarily arises from contaminated poultry meats. However, the genes expressed *in vivo* of the interaction between chicken and *C. jejuni* have not been screened. In this regard, *in vivo*-induced antigen technology (IVIAT) was applied to identify expressed genes *in vivo* during interaction between chicken and *C. jejuni*, a prevalent foodborne pathogen worldwide. Chicken sera were obtained by inoculating *C. jejuni* NCTC 11168 into Leghorn chickens through oral and intramuscular administration. Pooled chicken sera, adsorbed against *in vitro*-grown cultures of *C. jejuni*, were used to screen the inducible expression library of genomic proteins from sequenced *C. jejuni* NCTC 11168. Finally, 28 unique genes expressed *in vivo* were successfully identified after secondary and tertiary screenings with IVIAT. The genes were implicated in metabolism, molecular biosynthesis, genetic information processing, transport, regulation and other processes, in addition to Cj0092, with unknown function. Several potential virulence-associated genes were found to be expressed *in vivo*, including *chuA*, *flgS*, *cheA*, *rplA*, and Cj0190c. We selected four genes with different functions to compare their expression levels *in vivo* and *in vitro* using real-time RT-PCR. The results indicated that these selected genes were significantly upregulated *in vivo* but not *in vitro*. In short, the expressed genes *in vivo* may act as potential virulence-associated genes, the protein encoded by which may be meaningful vaccine candidate antigens for campylobacteriosis. IVIAT provides an important and efficient strategy for understanding the interaction mechanisms between *Campylobacter* and hosts.

**Keywords:** *Campylobacter jejuni*, *in vivo*-induced antigen technology (IVIAT), host-pathogen interaction, chicken

## Introduction

*Campylobacter jejuni* is a thermotolerant and microaerophilic spiral, gram-negative bacterium [26] and has emerged as the leading cause of human bacterial foodborne diarrheal disease worldwide. Children are especially susceptible to *C. jejuni* both in developed and developing countries [39, 41]. *C. jejuni* can colonize in the intestine of chicken and wild birds. The Guillain-Barré syndrome (GBS) is an acute polyneuropathy [18, 26] with a global incidence of 0.6–4 cases per 100,000 people. This disease generally occurs after infection by *C. jejuni* from contaminated food or water [1, 34, 35, 39]. Evidence suggests that GBS results from molecular mimicry between GM1 and GD1a gangliosides and the lipooligosaccharides (LOS) of *C. jejuni* [4, 27]. There is very great pressure to control human campylobacteriosis owing to the broad consumption of contaminated poultry meat products. To prevent chicken products contamination from *C. jejuni*, a better understanding of the biological characteristics of this pathogen, particularly the interaction mechanisms with hosts, is needed [4].

*C. jejuni* strain NCTC 11168 has become a widely used laboratory model for studying *Campylobacter* pathogenicity [5], and its genome sequence was annotated in 2000 [26]. Unlike other diarrhea-causing bacteria, *C. jejuni* does not express many classical virulence factors, such as pilus

structures [26, 41]. However, *C. jejuni* contains *O*- and *N*-linked glycosylation systems. It is worth mentioning that interactions between host and pathogen possibly play an important role in *Campylobacter* pathogenicity [26, 41].

*In vivo*-induced antigen technology (IVIAT) is a novel screening technique that identifies virulence-associated factors during host infection by pathogens [10, 29]. Using IVIAT, we can identify pathogenicity-related genes, which are expressed *in vivo* when a strong interaction occurs between the host and the microbe; however, some factors without immunogenicity are not detected [11, 29]. Screening can be performed using IVIAT without an ideal animal model for infection. Thus, this method is well suited for identifying virulence genes from *C. jejuni* [30]. The adsorption of convalescent sera during the application of IVIAT is very important when using a pathogen grown in the laboratory culture environment. If this step is conducted incompletely, false-positive clones can occur [21, 29]. To date, IVIAT has been used for a number of pathogens, including *Escherichia coli* [19, 21], *Vibrio* [11, 42], *Streptococcus* [9, 31, 40], *Salmonella* [12, 15, 23], *Mycobacterium* [6, 33], *Brucella* [24], and *Bacillus anthracis* [30].

In this study, a genomic expression library was constructed using sequenced *C. jejuni* NCTC 11168, and *in vivo*-induced antigens were identified from chicken inoculation antisera after being thoroughly adsorbed by cultures of *C. jejuni in vitro*. These screen of genes expressed *in vivo* may contribute to understanding the interaction between hosts and microbes, and several antigens are candidate vaccine sites and diagnostic markers.

## Materials and Methods

### Bacteria and Culture Conditions

*C. jejuni* NCTC 11168 was originally isolated from a clinical patient in the United Kingdom. This strain was cultured on Campy-blood free selective medium (CCDA) (Oxoid Ltd., Basingtoke, UK) plates at 42°C under microaerobic conditions for 30 h. *E. coli* DH5α and BL21 (DE3) cells were used as cloning and expression host strains, respectively, to generate the genomic expression library. These strains were inoculated in LB medium and cultivated at 37°C overnight. The BL21 (DE3) cells containing recombinant plasmids were plated on LB medium plus 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and kanamycin (kan) (50 μg/ml) and incubated at 30°C for library expression. For determining the protein expression profiles of *in vivo*-induced genes, the *C. jejuni* NCTC 11168 strain was cultured on CCDA medium plus 0.1% sodium deoxycholate (DOC; Sigma, USA) [25] (referred to as DOC plates).

### Recombinant Expressed Proteins

Proteins FlaA370 and CjaA were constructed and expressed in our laboratory. FlaA370 corresponds to the N-terminal 370 aa of FlaA, and its corresponding gene was inserted into the plasmid pET30a. CjaA was encoded from the complete *cjaA* gene, which was inserted into the pET30a plasmid. These two recombinant proteins were expressed and purified using affinity chromatography with a His Bind Purification kit (Novagen, Germany). The flagella of *C. jejuni* NCTC 11168 were extracted and purified according to Ibrahim *et al*. [17]. Aliquots of the proteins were stored at −80°C.

### Library Construction

To construct the genomic expression library of *C. jejuni* strain NCTC 11168, its genomic DNA was extracted (DNeasy Blood Tissue Kit; Qiagen, Germany) and then partially and randomly digested by *Sau*3A 1 (Promega, USA). These fragments, ranging from 0.4−3 kb, were then purified (Agarose Gel DNA Purification Kit ver. 2.0; TaKaRa, China) on a 0.9% agarose gel and separately ligated into pET30a, pET30b, and pET30c vectors, which had been digested by *Bam*HI 1 and dephosphorylated by alkaline phosphatase (Shrimp) (TaKaRa). The plasmids were then transformed into competent *E. coli* DH5α cells. After being cultured at 37°C overnight, the recombinant plasmids were extracted (MiniBest Plasmid Purification Kit ver. 2.0; Takara) and purified from *E. coli* DH5α. Aliquots of the plasmids were stored at −80°C for transforming into the BL21 (DE3) expression host strain.

### Preparation of Antisera

The chicken sera were obtained by inoculating *C. jejuni* NCTC 11168 into Leghorn chickens through oral and intramuscular administration, respectively. Prior to challenge, all the chickens were negative for *C. jejuni*-specific antibodies, as determined by an indirect ELISA test. Leghorn chickens ($n = 14$, 50 days old) were randomly grouped into two units (7/unit, named as groups 1 and 2). These chickens were challenged with 2 ml of strain NCTC 11168, intramuscularly for group 1 ($4 \times 10^9$ CFU), and orally for group 2 ($3 \times 10^8$ CFU), respectively. All animal experiments were conducted in accordance with national guidelines and were approved by the office of laboratory animal, Yangzhou University and Jiangsu administration committee of laboratory animals. The levels of the total antibodies of chicken were determined with indirect ELISA methods (developed by our laboratory). Chicken individual sera were combined in equal volume and adsorbed using *C. jejuni* NCTC 11168 whole cells cultured on CCDA plates on a rocking platform (Incubator Shaker; Crystal, China) at 4°C for 1 h. The sera were adsorbed with *C. jejuni* whole cells, *E. coli* BL21 (DE3) whole cells, ultrasonic lysis of *C. jejuni* or *E. coli* BL21 (DE3) cells, inactivated ultrasonic lysis supernatants of NCTC 11168 or BL21 (DE3) cells, secreted protein from NCTC 11168, and lysis-induced BL21 (DE3) cells. Indirect ELISAs were conducted for assessing the adsorption of sera by coating *C. jejuni* NCTC 11168 and *E. coli* BL21 (DE3) whole cells, lysates, CjaA, FlaA370

recombinant protein, and extracted flagellum protein. Dot-ELISA was conducted according to the standard protocol for assessing the reactivities of antibodies with *C. jejuni* whole-cell lysis.

### *In Vivo*-Induced Antigen Screening

The IVIAT screening was performed based on previously described methods [11, 21, 29] with a few modifications. An aliquot of library was transformed into *E. coli* BL21 (DE3) cells containing 50 μg/ml kan, and incubated at 37°C overnight. Library colonies were lifted onto sterile NC membranes, and incubated on kan/LB plates for 5 h at 37°C. These membranes with lifted colonies (colony side up) were overlaid on fresh kan/LB plates containing 1 mM IPTG, and incubated for 5 h at 30°C to induce expression of the cloned inserts. The NC membranes were removed, and were exposed in chloroform vapors for 15 min in a hermetic container. The membranes were blocked with 1% BSA and allowed to incubate for 1 h at room temperature. The membranes were washed three times in PBS plus 0.1% Tween-20 (PBST) for 30 min with gentle agitation at room temperature. Next, the membranes were incubated with 1:1,000 (v/v) dilution of adsorbed sera. Positive colonies were detected using goat anti-chicken-horseradish peroxidase (HRP) secondary antibodies and were visualized using a chemiluminescent ECL substrate (Thermo, USA). Positive clones identified during the primary screening were chosen from the master plates and subjected to secondary screening by patching in an alternating pattern with the BL21 (DE3) vector control. Plasmids from positive clones were subjected to DNA sequencing using pET30-specific primers.

### Functional Prediction of the Antigens Identified by IVIAT

The sequences of the *in vivo*-induced genes were analyzed by BLASTx alignment analysis and a Conserved Domain Database (CDD) domain search (http://www.ncbi.nlm.nih.gov/BLAST/). The subcellular localization of the putative IVIAT-identified proteins was predicted using PSORTb ver. 2.0.4 (http://www.psort.org). Functional classification was based on published studies of identified proteins in *C. jejuni* NCTC 11168 [26]. When no published functional information for *C. jejuni* was available, protein functional classification was based on predictive models using the Clusters of Orthologous Groups database (http://www.ncbi.nlm.nih.gov/COG/) or the Pfam database (http://www.sanger.ac.uk.Software/Pfam).

### Gene Expression Analysis by Quantitative Real-Time PCR

Gene expression profiles were tested by comparing the RNA transcription on DOC and CCDA plates with the expression of virulence induced with sodium deoxycholate [16, 25]. Total RNA was isolated using an RNAprep bacterial kit (TaKaRa) according to the manufacturer's instructions after a 15-h culture of strain NCTC 11168 on CCDA or DOC plates. The cDNA was synthesized using a RT-PCR kit (TaKaRa) according to the manufacturer's instructions with 500 ng of RNA. Real-time RT-PCR amplification of 2.0 μl of cDNA was performed using a reaction mixture containing SYBR Premix Ex Taq II (TaKaRa), 10 μM forward primer, 10 μM reverse primer, and diethyl pyrocarbonate-treated water. The real-time RT-PCR analysis was performed using a Gene Amp 7500 thermocycler (Applied Biosystems, CA, USA) with the following PCR parameters: 2 min at 50°C, followed by 40 cycles of denaturation at 95°C for 30 sec and annealing at 60°C for 34 sec. Four IVI genes (primers are shown in Table 1) were selected, and their transcription levels in the NCTC 11168 strain cultured on DOC plates were compared with those on CCDA plates. Gene Cj0402 was used to normalize these samples because this housekeeping gene is expressed both on DOC and CCDA plates [25]. Duplicate reactions were performed, and three biological replicates were used for each sample. Threshold cycle values were determined using 7500 software ver. 2.0.1 (Applied Biosystems).

**Table 1.** Primers used for real-time RT-PCR analysis.

| Primers | Genes | Sequence(5′–3′) |
|---|---|---|
| Cj0475-F | *rplA* | TGTTGTTTTACCTGCTGGAACGG |
| Cj0475-R | | CGCTACCCACAATATCTGCACCA |
| Cj0284c-F | *cheA* | GAGGGAGAGTCTCCTGTTGTGGC |
| Cj0284c-R | | TTTGTTGTTTGTTTCTTTTGTGCG |
| Cj0006-F | Cj0006 | GCCATTGCCGCAGCGAT |
| Cj0006-R | | AAGCACAAGCAACCGCTCTAC |
| Cj0663-F | *hslV* | TCTGGTACAGGCGATGTGGTC |
| Cj0663-R | | TCGGTATGTTTTGCTAAGGCTCTA |
| Cj0402-F | *glyA* | CGATGGAACGGATAATCACC |
| Cj0402-R | | AATACCTGCATTTCCAAGAGC |

### Western-Blotting for Expression and Reaction of IVI Genes

To confirm the immunoreactivity of the IVI proteins, 11 partial genes from recombinant plasmids of the NCTC 11168 genetic expression library were induced to express as His-tagged fusion proteins. The expression of 11 proteins in *E. coli* BL21(DE3) was induced with 1 mM IPTG at 37°C. Immune responses to these proteins in completely adsorbed chicken serum samples were evaluated using western blotting. In this study, serum was used at a 1:1,000 dilution. The immune responses to peroxidase-conjugated goat anti-chicken IgG at a dilution of 1:1,500 and a chemiluminescent ECL substrate (Thermo) were used for detection.

## Results

### Serum Selection and Adsorption

The higher titer sera (≥1:15,000) were collected and pooled from 14 individual chickens for screening the genomic expression library. The sera were detected after each adsorption process by indirect ELISA plates coated with *C. jejuni* NCTC 11168 and *E. coli* BL21 (DE3) whole cells, lysates, CjaA, FlaA370 recombinant protein, and extracted

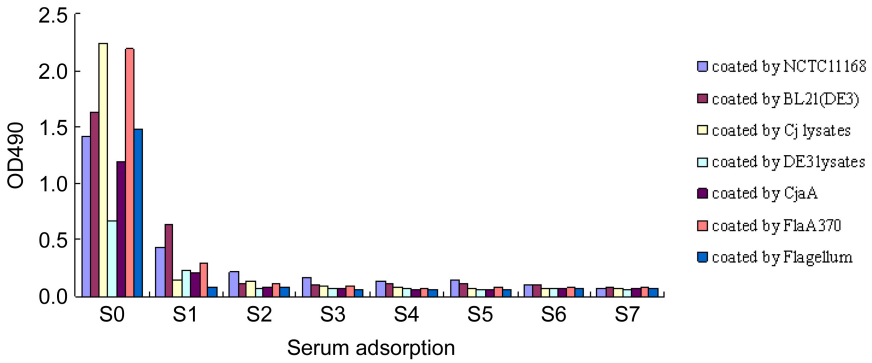


**Fig. 1.** Results of ELISA for chicken sera with seven coated antigens after each adsorption step.
The whole cells of *C. jejuni* NCTC 11168, whole cells of *E. coli* BL(DE3), lysates from *C. jejuni*, lysates from BL21(DE3), recombinant CjaA, FlaA370 protein, and extracted flagellum protein were used as coating antigens for assaying the adsorption of sera.

flagellum protein (Fig. 1). The immunoreactivity of the pooled sera with *in vitro*-grown *C. jejuni* antigen decreased progressively with a series of adsorption, and the immunoreactivity dropped clearly after the first adsorption step. Dot-ELISA results showed that unadsorbed chicken sera strongly reacted with *C. jejuni* whole-cell antigens. There were no spots on the NC membrane because the sera had been thoroughly adsorbed using *in vitro* antigens (Fig. 2). These data indicated that antibodies specific to *in vitro* antigens had been completely eliminated from the chicken sera set.

### Identification of *C. jejuni* Antigens by IVIAT

The *C. jejuni* NCTC 11168 library in *E. coli*, represented by a total of approximately 52,700 clones, was screened using extensively adsorbed, pooled immune chicken serum. Indeed, 207 immunoreactive clones were identified after primary screening. Of the initial clones, 56 were robustly positive upon secondary screening. Finally, 28 unique protein-expressing clones that continuously showed a strong positive reaction with the adsorbed sera antibodies were ascertained by nucleotide sequencing and homology analysis. These 28 proteins have a defined or suspected role during interaction between chicken and *C. jejuni*. These proteins are implicated in metabolism, molecular biosynthesis, genetic information processing, transport, and regulation (Table 2).

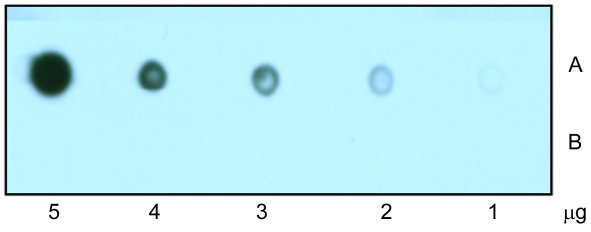


**Fig. 2.** Dot-ELISA results of reactivities of pooled unadsorbed (**A**) and adsorbed (**B**) chicken sera against *C. jejuni* whole-cell lysates.
The proteins of *C. jejuni* whole-cell lysates were quantified using UV spectrophotometry and diluted to 5 μg to 1 μg for each colony.

### Analysis of the *In Vivo* Gene Expression Profiles

To evaluate the *in vivo* expression of *C. jejuni* genes identified using the IVIAT method, we selected four genes according to their functional category to compare the expression levels *in vivo* and *in vitro* using real-time RT-PCR. An *in vitro* DOC plate culturing method was used for analyzing the RNA transcription, mimicking *in vivo* conditions. The real-time PCR results showed that these four genes were drastically upregulated under *in vivo*-like conditions (>1-fold), while the other five genes were not (Fig. 3).

### Evaluation of Immune Responses Between IVI Proteins and Adsorbed Serum

To evaluate the immune responses between IVI proteins and adsorbed serum, we selected 11 genes according to their functional category to express their products. The results of western blotting between the 11 IVI proteins and adsorbed chicken serum revealed that all there was only one specific band for 11 induced antigens (Fig. 4). There were specific immune responses between IVI proteins and chicken adsorbed serum.

**Table 2.** Twenty-eight genes identified by IVIAT in *C. jejuni* from chicken host.

| Gene (ORF) | Function of gene product | Predicted cellular location |
|---|---|---|
| Metabolism | | |
| *pheA* (Cj0316) | Chorismate mutase/prephenate dehydratase | Cytoplasmic |
| *fabH* (Cj0328c) | 3-Oxoacyl-ACP synthase | Cytoplasmic |
| *hemL* (Cj0853) | Glutamate-1-semialdehyde aminotransferase | Cytoplasmic |
| *leuC* (Cj1717c) | Isopropylmalate isomerase large subunit | Cytoplasmic |
| *panC* (Cj0297c) | Pantoate-beta-alanine ligase | Cytoplasmic |
| Cj0160c | Radical SAM domain protein | Cytoplasmic |
| Cj1476c | Pyruvate-flavodoxin oxidoreductase | Cytoplasmic |
| Molecular biosynthesis | | |
| *hslV* (Cj0663c) | ATP-dependent protease peptidase subunit | Cytoplasmic |
| Cj1200 | NLPA family lipoprotein | Cytoplasmic membrane |
| Genetic information processing | | |
| *rplA* (Cj0475) | 50S Ribosomal protein L1 | Cytoplasmic |
| *rpmH* (Cj0961c) | 50S Ribosomal protein L34 | Cytoplasmic |
| *aspS* (Cj0640c) | Aspartyl-tRNA synthetase | Cytoplasmic |
| *recG* (Cj0464) | ATP-dependent DNA helicase RecG | Cytoplasmic |
| *gatA* (Cj1059) | Aspartyl/glutamyl-tRNA amidotransferase subunit A | Cytoplasmic |
| *ileS* (Cj1061c) | Isoleucyl-tRNA synthetase | Cytoplasmic |
| Cj1481c | Recombination protein RecB | Cytoplasmic |
| Transport | | |
| *chuA* (Cj1614) | Hemin uptake system outer membrane receptor | Outer membrane |
| *corA* (Cj0726c) | Magnesium and cobalt transport protein CorA | Cytoplasmic membrane |
| Cj1106 | Periplasmic thioredoxin | Unknown |
| Regulator | | |
| *flgS* (Cj0793) | Signal transduction histidine kinase | Cytoplasmic membrane |
| Cj0006 | $Na^+/H^+$ antiporter family protein | Cytoplasmic membrane |
| Cj0497 | Lipoprotein, signal | Cytoplasmic |
| Cj0610c | Periplasmic protein | Unknown |
| Cj0190c | Hypothetical protein Cj0190c | Cytoplasmic |
| Cj0571 | Transcriptional regulator | Cytoplasmic |
| Others | | |
| *cheA* (Cj0284c) | Chemotaxis protein cheA | Cytoplasmic |
| Cj8486_0361 | Putative flagellar motility protein | Unknown |
| Unknown | | |
| Cj0092 | Periplasmic protein | Unknown |

## Discussion

*C. jejuni* is a major environmental microorganism and is ubiquitously found in hosts and media, including livestock and wild animals, and environmental water and soil [7]. Humans can be infected through the consumption of chicken products contaminated by *C. jejuni.* To control and eliminate campylobacteriosis, we must first understand the mechanisms of interaction between chicken and *C. jejuni.* In this study, IVIAT was employed to investigate the virulence-associated genes *in vivo.* IVIAT is a rigorous method that can identify virulence-associated antigens, which are expressed instantaneously *in vivo* when hosts are infected by pathogenic bacteria [29]. The sera of immunized

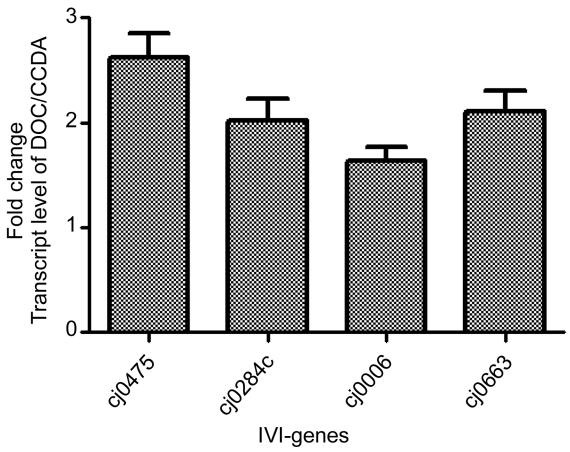


**Fig. 3.** *In vivo* gene expression relative to the level of expression *in vitro* by real-time PCR.
*C. jejuni* NCTC 11168 was cultured in plates of CCDA (control *in vitro*) and DOC (*in vivo*-like condition) for 15 h, and the transcription levels of mRNA were assessed by real-time PCR. The levels of transcription above 1-fold were considered upregulated in contrast with *in vitro*.

chicken were collected and used for IVIAT. Before IVIAT, individual serum titers of chickens were determined by indirect ELISA. The higher titer sera were selected for adsorption. To thoroughly remove the antibodies *in vitro*, we adsorbed the pooled serum six times using various antigen preparations, such as those involving *C. jejuni* and DE3 (BL21) whole cells and lysates. In this study, an expression library was generated from 52,700 clones, equating to 4 times the size of the NCTC 11168 genome. We selected several genes, including *chuA*, *flgS*, and Cj0190c, through IVIAT that were found to have products that were upregulated in the *C. jejuni* F38011 strain in the presence of 0.1% sodium DOC. These culture conditions have been reported to mimic the *in vivo* environment [25].

Seven *in vivo*-induced genes classified with a metabolism function were identified in this study and may have important roles in bacterial growth or pathogenicity *in vivo*. Previous studies [14] have shown that some strains of *C. jejuni* can utilize glutamine or glutathione to enhance its ability to colonize the intestines of hosts. We discovered that *hemL* (involved in glutamine or glutathione and asparagine metabolism) is expressed during chicken and human infection with *C. jejuni*, indicating that these genes may be correlated with bacterial colonization. The gene *fabH* (Cj0328c) was upregulated (3.09-fold by microarray) during chicken colonization, indicating that the protein encoded by *fabH* may be an important virulence-associated factor *in vivo* [36].

Two genes, including *hslV* (Cj0663c) and Cj1200, were classified as having a molecular biosynthesis function. Seven *in vivo*-induced genes (*rplA*, *rpmH*, *aspS*, *recG*, *gatA*, *ileS*, and Cj1481c) were assigned to a genetic information processing function, but their specific functions have not been determined. All bacteria have DNA repair mechanisms, which act to reduce DNA damage and to maintain genetic structures and stability [8]. For *C. jejuni*, genomic polymorphisms are important for adaptation to diverse environments. However, DNA repair is essential for its survival in response to significant environmental stress. We hypothesize that these DNA repair genes containing nine *in vivo*-associated factors may be significant when *C. jejuni* lives in human or chicken tissues.

We identified three virulence-associated genes, *chuA* (Cj1614), *corA* (Cj0726c), and Cj1106, which were assigned to a transportation function. Gene *chuA*, which encodes the outer membrane uptake protein hemin [32], exhibited highly upregulated transcription in an *in vitro* microarray,

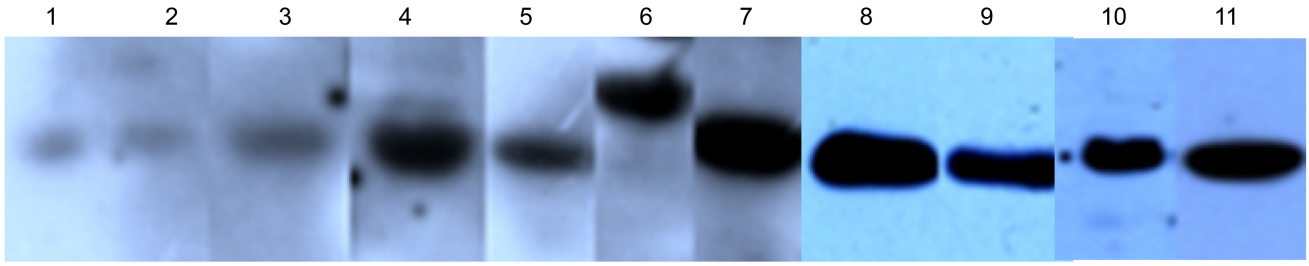


**Fig. 4.** Western blotting for expression and reaction of IVI genes.
Eleven IVI genes, including of 1-*aspS* (24-KD), 2-*recG* (24-KD), 3-*gatA* (24-KD), 4-*Cj0190c* (30-KD), 5-*chuA* (20-KD), 6-*hemL* (28-KD), 7-*panC*(20-KD), 8-*cheA* (14-KD), 9-*Cj0160c* (20-KD), 10-*pheA* (40-KD), and 11-*Cj1481* (25-KD), were expressed in *E. coli* BL21(DE3). The responses of the chicken serum samples to these proteins were visualized by western blotting. There was only one specific band for 11 induced antigens with adsorbed chicken serum.

indicating that *chuA* may be an essential factor for chicken colonization [36]. There are no reports on *corA*, which encodes for a magnesium and cobalt transport protein [3].

Six genes were obtained, and their products participate in regulation when chickens are infected with *C. jejuni*. One of the interesting invasion-associated genes [28], *flgS* (Cj0793), may encode a signal transduction histidine kinase in *C. jejuni*. Joslin and Hendrixson [2, 20] proposed that the FlgS histidine kinase may receive a signal from the formation or activity of the flagellar T3SS, which activates FlgR for the expression of flagellar rod and hook genes. His141 of FlgS is essential for autophosphorylation and phosphorelay of FlgR. These molecular modifications are the basis for the regulation and motility of the FlgSR two-component regulatory system [20, 37].

The gene *cheA* (Cj0284c), which encodes the chemotaxis protein CheA, is one of the fundamental components of the chemotaxis signaling pathway [13]. CheA plays a key role in chemotaxis by promoting CheY phosphorylation. The *C. jejuni cheA* mutants were nonchemotactic *in vitro* and showed defects in the colonization of a mouse model, suggesting a major function for CheA in chemotaxis in the presence of infection [22, 38].

In short, IVIAT identified 28 *C. jejuni* NCTC 11168 genes expressed *in vivo* during interaction between chicken and *C. jejuni*. Several genes are closely correlated with the virulence and pathogenesis of *C. jejuni*, and these antigens may be candidates for vaccines.

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