(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 August 2018 (02.08.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/140717 A1

(51) International Patent Classification:
A61K 39/02 (2006.01) *C12N 15/74* (2006.01)
C07K 14/205 (2006.01)

(21) International Application Number:
PCT/US2018/015438

(22) International Filing Date:
26 January 2018 (26.01.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/451,146 27 January 2017 (27.01.2017) US

(71) Applicant: **UNIVERSITY OF FLORIDA RESEARCH FOUNDATION INCORPORATED** [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).

(72) Inventors; and
(71) Applicants: **CURTISS, III, Roy** [US/US]; 2312 N.W. 23rd Terrace, Gainesville, FL 32605-2807 (US). **WANDA, Soo-Young** [KR/US]; 2801 NW 23rd Blvd., Apt. 129, Gainesville, FL 32605 (US).

(74) Agent: **EISENSCHENK, Frank, C.** et al.; Saliwanchik, Lloyd & Eisenschenk, P.O. Box 142950, Gainesville, FL 32614-2950 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2018/140717 A1

(54) Title: A FOOD SAFETY VACCINE TO CONTROL *SALMONELLA ENTERICA* AND REDUCE *CAMPYLOBACTER* IN POULTRY

(57) Abstract: Described herein are compositions and methods for making and using recombinant bacteria that are capable of regulated attenuation, regulated expression of one or more antigens of interest, and/or N-glycan modification of secreted/surface antigens.

A FOOD SAFETY VACCINE TO CONTROL *SALMONELLA ENTERICA* AND REDUCE *CAMPYLOBACTER* IN POULTRY

GOVERNMENTAL RIGHTS

[0001] This invention was made with government support under Grant No. 12229724 awarded by the National Institute of Food and Agriculture of the United States Department of Agriculture. The government has certain rights in the invention.

RELATED APPLICATION

[0002] This application claims priority to U.S. Provisional Application No. 62/451,146, filed on January 27, 2017. The entire content of the foregoing application is expressly incorporated herein by reference.

BACKGROUND

[0003] Foodborne human pathogens are a major public health problem. In particular, *Campylobacter* and *Salmonella* are leading causes of bacterial foodborne disease worldwide. *Campylobacter* gastroenteritis is associated with poor handling of raw chicken or consumption of undercooked chicken. (1)(2) Domestic poultry supply, including chickens, turkeys, ducks, and geese, is frequently infected with *C. jejuni* and *C. coli*. (3) *Salmonella* human infections are associated, in part, with the ingestion of foods contaminated by animal feces or cross-contaminated by other sources, including poultry. (4)(5) The increasing consumption of poultry and poultry products has rendered foodborne diseases associated with poultry a significant public health concern.

[0004] Strategies for controlling *Campylobacter* and *Salmonella* in poultry include the administration of antibiotics. However, the overuse of antibiotics and the risk of developing antibiotic-resistant bacterial strains has decreased interest in the use of antibiotics for these purposes. Thus, poultry vaccination has emerged as an important strategy to mitigate the prevalence of at least these two foodborne pathogens in poultry supplies.

[0005] Recombinant attenuated *Salmonella* vaccines (RASVs) have been developed as vaccines and antigen delivery systems to stimulate protective immune responses against a multitude of antigens. Improved RASVs that are engineered to include inducible promoter-regulated pathogenic attributes have been recently developed. Improvements in this technology has included the development of Regulated Delayed Attenuated RASVs (RDA

RASVs) which are grown *in vitro* in the presence of an inducer (*e.g.*, a sugar) to induce the expression of genes associated with pathogenesis. Upon administration to a subject, the RDA RASVs replicate with full virulence and colonize lymphoid tissues to induce potent immune responses. However, as the levels of inducer decreases within the subject and after multiple rounds of replication, the RDA RASVs become attenuated, thereby preventing further proliferation of the bacteria *in vivo*.

[0006] The use of RASVs for the prophylaxis and treatment of foodborne pathogens in poultry is particularly promising. However, new means to enhance the immunogenicity and safety of the vaccines *in vivo* are necessary. RASVs and pharmaceutical compositions capable of inducing potent immune responses to multiple foodborne pathogens in poultry are particularly desirable.

SUMMARY

[0007] The instant disclosure provides strains of recombinant bacteria, including *Salmonella*, with enhanced immunogenic properties and desirable safety features. The recombinant bacteria can be safely used to effectively deliver antigenic compounds to a subject (*e.g.*, poultry) in order to mount potent immunogenic responses against pathogens such as *Campylobacter and Salmonella*. These strains deliver multiple conserved protective *Salmonella* surface/secreted antigens and/or *Campylobacter* N-glycan modified surface/secreted antigens to induce protective immunity.

[0008] In some embodiments, disclosed herein is a recombinant derivative of a pathogenic bacterium, comprising regulated-delayed attenuation, a regulated-delayed synthesis of an antigen of interest, a regulated-delayed lysis *in vivo* phenotype, and synthesizing and delivering one or more protein antigens of *C. jejuni*.

[0009] In some embodiments, the recombinant bacteria described herein are capable of synthesizing and attaching a *Campylobacter* N-glycan to an antigen of interest in order to enhance the immunogenicity of the antigen *in vivo*. The recombinant bacteria may depend on two or three sugars to regulate the virulence phenotype of the bacteria by controlling the expression of multiple virulence genes and, optionally, the antigen of interest, as well as a regulated delayed lysis phenotype, allowing for biological containment and the enhancement of immunogenic properties. The dependence on multiple sugars enhances the safety of the recombinant bacteria, given the improbability that the organisms will encounter all sugars in

a naturally-occurring environment. See PCT/US18/14860, filed on January 23, 2018, the entire contents of which are expressly incorporated herein by reference.

[0010] Specifically, the bacteria disclosed herein are based upon a recombinant attenuated *Salmonella* vaccine (RASV) derived from the highly avian virulent *S.* Typhimurium UK-1 strain and can be used to synthesize and deliver multiple *Campylobacter jejuni* conserved protective protein antigens. Vaccination with these RASVs have been found to induce immunity against multiple *Salmonella* serotypes and *C. jejuni* infecting poultry. The instant disclosure further provides an innovative new RASV design strategy to generate these food safety vaccines that induce superior levels of protective immunity to several poultry pathogens. These RASV vectors were programed to undergo regulated delayed lysis in various cell compartments within the immunized animal to induce superior mucosal and systemic antibody and cellular immunities. In addition, these live RASVs display complete biological containment with no persistence *in vivo* and no survival if excreted. Widespread use of these RASVs is predicted to substantially reduce *Salmonella* and *C. jejuni* infections and ultimately eliminate *C. jejuni* colonization of chickens to reduce transmission of these pathogens through the food chain to humans. Furthermore, the use of these much improved vector systems will also decrease use of antibiotics during poultry husbandry and thus reduce the selective pressure for drug-resistant bacterial species that can also be transmitted through the food chain to humans.

[0011] The instant disclosure further provides a validated and highly immunogenic *S.* Typhimurium UK-1 RASV that displays regulated delayed attenuation, regulated delayed synthesis of recombinant antigens and regulated delayed lysis phenotypes. Derivatives were constructed that are enabled to synthesize and deliver multiple *C. jejuni* protein antigens. Thus, because of the RASV's inability to synthesize the serotype-specific LPS O-antigen *in vivo* to expose the LPS core that is the same in all, *Salmonella* serotypes induce cross protective immunity to most *Salmonella* serotypes while also delivering multiple *C. jejuni* protein antigens to induce superior protective immunity against *C. jejuni*.

[0012] The RASVs constructed were designed to display at the time of course spray or oral delivery to poultry the same attributes as the wild-type virulent UK-1 parent strain to contend with host defense strategies, and successfully invade through mucosal surfaces to efficiently colonize internal effector lymphoid tissues before displaying the attenuation phenotype, and thus the inability to induce any disease symptoms or impair or reduce growth. The RASVs reaching these internal tissues were found to gradually commence to

serve as factories to synthesize protective *C. jejuni* antigens to be delivered by secretion and ultimately by lysis of the RASV. Since lysis is enabled to occur in diverse extracellular and intracellular compartments, desired mucosal, systematic antibody and cellular immune responses were induced. These innovative strategies provide a classical means of attenuation used previously that reduced immunogenicity because of impaired colonizing abilities of attenuated strains. In addition, these RASVs with the regulated delayed lysis phenotype display completed biological containment with no persistence of vaccine cells *in vivo* and no survivors if excreted.

[0013] In one aspect, disclosed herein is a recombinant derivative of a pathogenic bacterium comprising a regulated-delayed attenuation phenotype; a regulated-delayed expression of an antigen of interest; a regulated-delayed lysis *in vivo* phenotype; and which is capable of synthesizing and delivering one or more protein antigens of *C. jejuni*. In one embodiment, the bacterium further comprises mutations that cause display of the universal LPS core polysaccharide *in vivo*.

[0014] In one aspect, disclosed herein is a recombinant derivative of a pathogenic bacterium comprising a regulated-delayed attenuation phenotype; a regulated-delayed expression of an antigen of interest; a regulated-delayed lysis *in vivo* phenotype; and which is capable of synthesizing and delivering one or more protein antigens that display the *Campylobacter jejuni* N-glycan.

[0015] In one aspect, disclosed herein is a recombinant derivative of a pathogenic bacterium comprising a regulated-delayed attenuation phenotype; a regulated-delayed expression of an antigen of interest; a regulated-delayed lysis *in vivo* phenotype; and which is capable of synthesizing and delivering one or more protein antigens of *C. jejuni* and also displays the *Campylobacter jejuni* N-glycan.

[0016] In one embodiment, the bacterium comprises one or more *Campylobacter pgl* operon genes. In one embodiment, the one or more *Campylobacter pgl* operon genes are codon-optimized for expression in the bacterium.

[0017] In one embodiment, the bacterium is a Gram-negative bacterium. In one embodiment, the bacterium belongs to the family *Enterobacteriaceae*.

[0018] In one embodiment, the antigen of interest is a *Campylobacter* antigen. In one embodiment, the *Campylobacter* antigen is an antigen selected from **Table 4**, or an antigen having homology or identity thereto, as described herein. In one embodiment, the antigen of interest is selected from the group consisting of Peb1, CjaA, Dps, TlyA, Omp18, Cj0998c,

Cj0034c, Cj0168c, Cj0248, Peb3, CmeC, Cj0404, Cj0420, Cj0427, Cj0428, PorA, FlaA, CadF, and Cj1656c.

[0019] In one embodiment, the *C. jejuni* N-glycan is attached to the antigen of interest.

[0020] In one embodiment, the bacterium comprises insertion of all or part of the *cj1433c* gene into a deletion of the *ompA* gene to generate a fusion of the Cj1433c protein.

[0021] In one embodiment, the regulated-delayed attenuation is conferred by the gene *fur* or *mntR*.

[0022] In one embodiment, the regulated-delayed lysis *in vivo* phenotype is conferred by ΔP_{murA}::TT *araC* P_{BAD} *murA*, Δ*asdA*::TT *araC* P_{BAD} *c2*, or Δ(*wza-wcaM*) deletions or deletion/insertion mutations.

[0023] In one embodiment, the bacterium further comprises a mutation in the *sifA* gene.

[0024] In one embodiment, the bacterium further comprises a mutation in the *relA* gene.

[0025] In one aspect, disclosed herein is a pharmaceutical composition comprising the recombinant bacterium, and a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical composition further comprises a second recombinant derivative of a pathogenic bacterium, wherein said bacterium comprises a nucleic acid encoding a second antigen of interest. In one embodiment, the second antigen of interest is a *Salmonella* antigen.

[0026] In one aspect, disclosed herein is a method for inducing protective immunity in an avian, the method comprising administering to the avian an effective amount of a pharmaceutical composition disclosed herein.

[0027] Other aspects and iterations of the invention are described more thoroughly below.

BRIEF DESCRIPTION OF THE FIGURES

[0028] **Figs. 1A** and **1B** depict proliferation assays of antigen-specific lymphocytes. In **Fig. 1A**, representative samples of splenic lymphocytes isolated from chickens immunized with the non-lysis vector control RASV χ11840(pYA3342) (G2), non-lysis RASV χ11442(pYA5301) (G3), non-lysis Δ*sifA* RASV χ11840(pYA5301) (G4), lysis vector control RASV χ11791(pYA3681) (G5), lysis RASV χ11730(pYA5293) (G6), and lysis Δ*sifA* RASV

χ11791(pYA5293) (G7), were labeled with CSFE and stimulated with the *Eimeria* SO7 antigen. The proliferative lymphocyte population was defined by CFSE dilution. **Fig. 1B** presents a graphic analysis of cell proliferation in each group described in **Fig. 1A**. Bar, mean, and SD are the sum of 3 independent experiments each using three chickens per group. Asterisks represent significant differences between the groups as indicated ($p < 0.05$). Also correlated with increased INF-γ and IL-2 production and increased CD4 and CD8 responses.

[0029] **Figs. 1C** and **1D** depict the induction of intestinal immunoglobulin A (IgA) against *E. tenella* SO7 in immunized chickens, either on day 35 post-immunization (**Fig. 1C**) or on day 42 post-immunization (**Fig. 1D**).

[0030] **Fig. 2A** depicts SO7-specific CD4 and CD8 proliferation analysis in non-lysis versus lysis RASVs without and with escape from SCV due to the Δ*sifA* mutation.

[0031] **Fig. 2B** depicts lymphocyte activation and cytokine secretion after *in vitro* homologous recall of splenic lymphocytes from chicks vaccinated with RASV vaccine strains.

[0032] **Fig. 3A** and **3B** depict lysis vector maps. **Fig. 3A -** Lysis vector pYA4763, pBR ori. **Fig. 3B** - Lysis vector pG8R17 with improved *bla* SS, pBR ori.

[0033] **Figs. 4A** and **4B** depict antigen productions in RASV strain χ12341.

[0034] **Fig. 5** depicts western blot analyses of *C. jejuni* antigens in χ12452 *S.* Typhimurium strain grown in L broth with 0.1% each of arabinose, rhamnose, and mannose with/without induction by 1mM IPTG. Monoclonal anti-poly-histidine antibody produced in mice was used for detection of antigens synthesized.

[0035] **Fig. 6** depicts the genetic map of the *C. jejuni pgl* operon.

[0036] **Fig. 7** depicts expression of the *pgl* operon with/without deletion of the *pglB* gene, with the addition of synthesized *C. jejuni* N-glycan to the LPS core in a *S.* Typhimurium with a deletion of the *wbaP* gene.

[0037] **Figs. 8A** and **8B** depict the structural aspects of the modified *S.* Typhimurium OmpA protein with the insertion of the Cj1433c gene encoded sequences, specifying the nine repeats to which the *C. jejuni* conserved N-glycan is attached as a consequence of the *C. jejuni pgl* operon and the activity of the *Salmonella* WaaL enzyme.

DETAILED DESCRIPTION

[0038] In order that the disclosure may be more readily understood, certain terms are first defined. These definitions should be read in light of the remainder of the disclosure and

as understood by a person of ordinary skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. Additional definitions are set forth throughout the detailed description.

[0039] As used herein, the term "recombinant bacterium" refers to a bacterial cell that has been genetically modified from its native state. For instance, a recombinant bacterium may comprise one or more nucleotide insertions, nucleotide deletions, nucleotide rearrangements, and nucleotide modifications. These genetic modifications may be introduced into the chromosome of the bacterium, or alternatively be present on an extrachromosomal nucleic acid (*e.g.*, a plasmid). Recombinant bacteria of the disclosure may comprise a nucleic acid located on a plasmid. Alternatively, the recombinant bacteria may comprise a nucleic acid located in the bacterial chromosome (*e.g.*, stably incorporated therein). In some embodiments, the recombinant bacterium is avirulent. In some embodiments the recombinant bacterium exhibits reduced virulence. In some embodiments, the recombinant bacterium is non-virulent. In some embodiments, the recombinant bacterium is attenuated. In another embodiment, the recombinant bacterium is a recombinant derivative of a pathogenic bacterium.

[0040] As used herein, the term "gene" refers to a nucleic acid fragment that encodes a protein or a fragment thereof, or a functional or structural RNA molecule, and may optionally include a regulatory sequence preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence of the nucleic acid. In some embodiments, a "gene" does not include regulatory sequences preceding and following the coding sequence.

[0041] In one embodiment, the gene is a heterologous gene. In another embodiment, the nucleic acid is a heterologous nucleic acid. As used herein, the terms "heterologous gene" or "heterologous nucleic acid" refer to a gene or a nucleic acid sequence present in a recombinant cell, *e.g.,* bacterium, that is not normally found in the wild-type cell, *e.g.,* bacterium, in nature. In some embodiments, the heterologous gene or heterologous nucleic acid is exogenously introduced into a given cell. In some embodiments, a heterologous gene may include a gene, or fragment thereof, introduced into a non-native host cell. In some embodiments, the term "heterologous gene" includes a second copy of a native gene, or fragment thereof, that has been introduced into the host cell in addition to the corresponding native gene. A heterologous nucleic acid may also include, in some embodiments, a gene sequence that is naturally-found in a given cell but which has been modified, *e.g.,* by

regulation by a different promoter sequence, to expresses an unnatural amount of the nucleic acid and/or the polypeptide which it encodes; and/or two or more nucleic acid sequences that are not found in the same relationship to each other in nature.

[0042] As used herein, the term "endogenous gene" refers to a native gene that is present in its natural location in the genome of an organism (*e.g.*, a bacterial chromosome).

[0043] A "promoter" as used herein, refers to a nucleic acid sequence that is capable of controlling the expression of a coding sequence or gene. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of a nucleic acid. For example, a promoter may include one or more nucleic acids that are specifically recognized by a transcriptional activator protein (*e.g.*, an enhancer element), a transcriptional repressor protein, a polymerase, and the like. The term "operably linked," as used herein, means that expression of a nucleic acid sequence is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) of the nucleic acid sequence under its control. The distance between the promoter and a nucleic acid sequence to be expressed may be approximately the same as the distance between that promoter and the native nucleic acid sequence it controls. As is known in the art, variation in this distance may be accommodated without loss of promoter function. The nucleic acid sequences of the promoters described herein are known in the art, and methods of operably-linking these promoters to a gene (*e.g.*, a gene encoding a repressor) are known in the art.

[0044] In some embodiments, the promoter for use as described herein may be regulated directly or indirectly by a sugar. For example, in some embodiments, the promoter is responsive to the level of arabinose, otherwise referred to herein as an "arabinose-regulatable promoter". Generally speaking, arabinose may be present during the *in vitro* growth of a bacterium, while typically absent from host tissue. In one embodiment, the promoter is derived from an *araC*-P_{araBAD} system from *Escherichia coli*. The *araC* P_{araBAD} system is a tightly regulated expression system, which has been shown to work as a strong promoter induced by the addition of low levels of arabinose. The *araC-araBAD* promoter is a bidirectional promoter controlling expression of the *araBAD* nucleic acid sequences in one direction, and the *araC* nucleic acid sequence in the other direction.

[0045] For convenience, the portion of the *araC-araBAD* promoter that mediates expression of the *araBAD* nucleic acid sequences, and which is controlled by the *araC* nucleic acid sequence product, is referred to herein as P_{araBAD}. For use as described herein, a

cassette with the *araC* nucleic acid sequence and the *araC-araBAD* promoter may be used. This cassette is referred to herein as *araC* P_{araBAD}. The AraC protein is both a positive and negative regulator of P_{araBAD}. In the presence of arabinose, the AraC protein is a positive regulatory element that allows expression from P_{araBAD}. In the absence of arabinose, the AraC protein represses expression from P_{BAD}. Other enteric bacteria contain arabinose regulatory systems homologous to the *araC-araBAD* system from *E. coli*, including, for example, S. Typhimurium. For example, the *E. coli* AraC protein only activates *E. coli* P_{araBAD} (in the presence of arabinose) and not *S.* Typhimurium P_{araBAD}. Thus, an arabinose-regulated promoter may be used in a recombinant bacterium that possesses a similar arabinose operon, without substantial interference between the two, if the promoter and the operon are derived from two different species of bacteria. Generally speaking, the concentration of arabinose necessary to induce expression is typically less than about 2% (w/w) in a culture media. In some embodiments, the concentration is less than about 1.5%, 1%, 0.5%, 0.2%, 0.1%, or 0.05% (w/w) in a culture media. In other embodiments, the concentration is 0.05% or below, *e.g.* about 0.04%, 0.03%, 0.02%, or 0.01% (w/w). In an exemplary embodiment, the concentration is about 0.05% (w/w) in a culture media.

[0046] In other embodiments, the promoter may be responsive to the level of maltose in the environment, otherwise referred to herein as a "maltose-regulatable promoter". In some embodiments, the recombinant bacteria described herein are cultured in a medium comprising maltose. The *malT* gene encodes MalT, a positive regulator of four maltose-responsive promoters (P_{PQ}, P_{EFG}, P_{KBM}, and P_S). The combination of *malT* and a *mal* promoter creates a tightly regulated expression system that has been shown to work as a strong promoter induced in the presence of maltose. Unlike the *araC*-P_{araBAD} system, *malT* expression is regulated by a promoter (*i.e.*, P_T) that is functionally unrelated to the other mal promoters. P_T is not regulated by MalT. The *malEFG-malKBM* promoter is a bidirectional promoter that controls expression of the malKBM nucleic acid sequences in one direction, and the *malEFG* nucleic acid sequences in the other direction. For convenience, the portion of the *malEFG-malKBM* promoter that mediates expression of the *malKBM* nucleic acid sequence, and which is controlled by MalT, is referred to herein as P_{malKBM}, and the portion of the *malEFG-malKBM* promoter that mediates expression of the *malEFG* nucleic acid sequence, and which is controlled by MalT, is referred to herein as P_{EFG}. Full induction of P_{KBM} requires the presence of the MalT binding sites of P_{malEFG}. For use in the vectors and systems described herein, a gene cassette comprising a nucleic acid sequence encoding MalT

and a *mal* promoter may be used. This gene cassette is referred to herein as *malT*-P_{mal}. In the presence of maltose, the MalT is a positive regulatory element that allows for expression mediated by P_{mal}. Generally speaking, the concentration of maltose necessary to induce expression is typically less than about 1% (w/w) in a culture media. In some embodiments, the concentration is less than about 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3% 0.2%, 0.1%, or 0.05% (w/w) in a culture media. In other embodiments, the concentration is 0.05% or below, *e.g.* about 0.04%, 0.03%, 0.02%, or 0.01% (w/w). In an exemplary embodiment, the concentration is about 0.2% to about 0.4% (w/w) in a culture media.

[0047] In still other embodiments, the promoter used herein is responsive to the level of rhamnose in the environment, otherwise referred to herein as a "rhamnose-regulatable promoter". Analogous to the *araC*-P_{araBAD} system described above, the *rhaRS*-P_{rhaB} activator-promoter system is tightly regulated by rhamnose. Expression from the rhamnose promoter (P_{rha}) is induced to high levels in the presence of rhamnose. In some embodiments, the bacteria are cultured in the presence of rhamnose. Rhamnose is commonly found in bacteria but rarely found in human subjects. The *rhaBAD* operon is controlled by the P_{rhaBAD} promoter. This promoter is regulated by two activators, RhaS and RhaR, and the corresponding nucleic acid sequences belong to one transcription unit that is located in the opposite direction of the *rhaBAD* nucleic acid sequences. In the presence of L-rhamnose, RhaR binds to the P_{rhaRS} promoter and activates the production of RhaR and RhaS. RhaS together with L-rhamnose, in turn, bind to the P_{rhaBAD} and the P_{rhaT} promoters and activates the transcription of the structural nucleic acid sequences. Full induction of the arabinose, maltose and rhamonse regulated promoters described herein requires binding of the Crp-cAMP complex, which is a key regulator of catabolite repression.

[0048] Although both L-arabinose and L-rhamnose act directly as inducers of the expression of regulons that mediate their catabolism, important differences exist in regard to the regulatory mechanisms. L-Arabinose acts as an inducer with the activator AraC in the positive control of the arabinose regulon. However, the L-rhamnose regulon is subject to a regulatory cascade, and is therefore subject to even tighter control than the *araC*-P_{araBAD} system. L-Rhamnose acts as an inducer with the activator RhaR for synthesis of RhaS, which in turn acts as an activator in the positive control of the rhamnose regulon. In the present disclosure, rhamnose may be used to interact with the RhaR protein and then the RhaS protein may activate transcription of a nucleic acid sequence operably-linked to the P_{rhaBAD} promoter.

[0049] In still other embodiments, the promoter may be responsive to the level of xylose in the environment, referred to herein as a "xylose-regulatable promoter". Generally, xylose concentrations of between 0.0002% to 0.63% (w/w) in the environment activate the expression of a xylose inducible promoter described herein. (6) The *xylR*-P_{xylA} system is another well-established inducible activator-promoter system. Xylose induces xylose-specific operons (*e.g.*, *xylE*, *xylFGHR*, and *xylAB*) which are regulated by XylR and the cyclic AMP-Crp system. The XylR protein serves as a positive regulator by binding to two distinct regions of the xyl nucleic acid sequence promoters. As with the *araC*-P_{araBAD} system described above, the *xylR*-P_{xylAB} and/or *xylR*-P_{xylFGH} regulatory systems may be used. In these embodiments, *xylR*-P_{xylAB} xylose interacting with the XylR protein activates transcription of nucleic acid sequences operably-linked to either of the two P_{xyl} promoters.

[0050] As used herein, the term "exogenous" refers to a substance (*e.g.,* a nucleic acid or polypeptide) present in a cell other than its native source. The term exogenous can refer to a nucleic acid or a protein that has been introduced by a process involving the hand of man into a biological system such as a cell or organism in which it is not normally found or in which it is found in undetectable amounts. A substance can be considered exogenous if it is introduced into a cell or an ancestor of the cell that inherits the substance. In contrast, the term "endogenous" refers to a substance that is native to the biological system or cell.

[0051] As used herein, the term "invasive" when used in reference to a bacterium refers to a bacterium that is able to be absorbed by an eukaryotic cell, or a bacterium that actively penetrates an eukaryotic cell. In some embodiments, an invasive bacterium penetrates an eukaryotic cell and reaches the eukaryotic cell cytoplasm by lysis of a vacuolar membrane.

[0052] As used herein, the term "pathogenic" when used in reference to a bacterium refers to a bacterium capable of infecting and causing disease in a host, as well as producing infection-related symptoms in the infected host. A bacterial species that is a pathogen and is pathogenic can be rendered attenuated or avirulent such that it no longer produces infection-related symptoms in the infected host. Such bacteria are referred to as "attenuated derivatives of pathogenic bacteria."

[0053] A "pharmaceutical composition," as used herein, refers to a composition comprising an active ingredient (*e.g.,* a recombinant bacterium described herein) with other components such as a physiologically suitable carrier and/or excipient.

[0054] As used herein, the term "pharmaceutically acceptable carrier" or a "pharmaceutically acceptable excipient" refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline (*e.g.,* phosphate-buffered saline (PBS)); (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C_2-C_{12} alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, disintegrating agents, binders, sweetening agents, flavoring agents, perfuming agents, protease inhibitors, plasticizers, emulsifiers, stabilizing agents, viscosity increasing agents, film forming agents, solubilizing agents, surfactants, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable excipient" or the like are used interchangeably herein.

[0055] A "plasmid" or "vector" includes a nucleic acid construct designed for delivery to a host cell or transfer between different host cell. The nucleic acid incorporated into the plasmid can be operatively linked to an expression control sequence when the

expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence.

[0056] As used herein, the terms “protein" and “polypeptide" are used interchangeably herein to designate a series of amino acid residues, connected to each other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms "protein", and "polypeptide" refer to a polymer of amino acids, including modified amino acids (*e.g.*, phosphorylated, glycated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. The terms "protein" and “polypeptide” as used herein refer to both large polypeptides and small peptides. The terms "protein" and "polypeptide" are used interchangeably herein when referring to a gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[0057] A “nucleic acid” or “nucleic acid sequence” may be any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double- stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable nucleic acid molecules are DNA, including genomic DNA or cDNA. Other suitable nucleic acid molecules are RNA, including mRNA, rRNA, and tRNA.

[0058] Alterations of the native amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion required. Techniques for making such alterations are very well established and include, for example, those disclosed by Walder et al. (7); Bauer et al. (8); Craik (9); Smith et al. (10) ; and U.S. Pat. Nos. 4,518,584 (11) and 4,737,462 (12), which are herein incorporated by reference in their entireties. Any cysteine residue not involved in maintaining the proper conformation of

the polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to the polypeptide to improve its stability or facilitate oligomerization.

[0059] The term "statistically significant" or "significantly" refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[0060] As used herein, the term “host cell” refers to a cell in an organism to which the recombinant bacterium is being administered in order to, for example, induce an immune response. In one embodiment, a host is a bird, equine, or human and a host cell refers, respectively, to a bird cell, an equine cell, or a human cell.

[0061] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean ±1%.

[0062] The articles “a” and “an,” as used herein, should be understood to mean “at least one,” unless clearly indicated to the contrary.

[0063] The phrase “and/or,” when used between elements in a list, is intended to mean either (1) that only a single listed element is present, or (2) that more than one element of the list is present. For example, “A, B, and/or C” indicates that the selection may be A alone; B alone; C alone; A and B; A and C; B and C; or A, B, and C. The phrase “and/or” may be used interchangeably with “at least one of” or “one or more of” the elements in a list.

[0064] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

I. Recombinant Bacteria

[0065] The present disclosure provides, in some embodiments, a recombinant bacterium capable of regulated expression of at least one nucleic acid sequence encoding an antigen of interest (*e.g.*, a *Campylobacter* antigen). The recombinant bacterium described herein is particularly effective in eliciting an immune response (*e.g.*, protective immunity) against the antigen of interest because the bacterium comprise multiple recombinant regulatory systems that permit the bacterium to replicate upon administration and to colonize

lymphoid tissues in a subject in order to elicit potent immune responses. However, after multiple replication cycles *in vivo*, the bacterium ultimately exhibits an attenuated phenotype which allows for safe administration to a subject, for example as a vaccine composition. The recombinant regulatory systems of the bacteria described herein depend, in part, on multiple genetic regulatory elements that are responsive to one or more sugars (*e.g.*, arabinose, rhamnose, mannose, maltose, xylose, and galactose) that not available to the bacterium *in vivo*. Thus, using the phenotype of the recombinant bacteria described herein can be altered upon administration to a subject. In some embodiments, the phenotype of the recombinant bacteria described herein is regulated-delayed expression of an antigen of interest, and the gene conferring the phenotype encodes an antigen of interest. In some embodiments, the subject is administered one or more sugars before, after or concurrently with the administration of a recombinant bacterium described herein in order to activate and/or repress a sugar-responsive regulatory system of the bacteria. In some embodiments, the recombinant bacterium described herein comprises at least three regulatory systems, each dependent on a different sugar, which facilitates initial invasion of a host cell in the subject, delayed attenuation, and improved immunogenicity.

[0066] In some embodiments, the recombinant bacterium described herein can be regulated for delayed attenuation *in vivo*. In some embodiments, the recombinant bacterium described herein is capable of regulated delayed expression of a nucleic acid encoding an antigen of interest. In some embodiments, the recombinant bacterium described herein exhibits regulated production of Generalized Modules for Membrane Antigens (GMMA) *in vivo*, which may lead to enhanced production of conserved outer membrane proteins present in the bacterium, and ultimately improved immunogenicity. In some embodiments, the recombinant bacterium described herein is capable of both regulated expression of at least one nucleic acid encoding at least one antigen of interest and regulated attenuation. In some embodiments, the recombinant bacterium described herein is capable of both regulated expression of at least one nucleic acid encoding at least one antigen of interest and regulated production of GMMA *in vivo*. In some embodiments, the recombinant bacterium described herein is capable of both regulated production of GMMA *in vivo*, and regulated attenuation. In some embodiments, the recombinant bacterium described herein is capable of regulated expression of at least one nucleic acid encoding at least one antigen of interest, regulated attenuation, and regulated production of GMMA *in vivo*. In some embodiments, each of

these properties is directly or indirectly regulated by the abundance of at least one sugar (*e.g.*, arabinose, rhamnose, mannose, xylose, maltose, and galactose).

[0067] In some embodiments, the bacterium described herein is a Gram negative bacterium. In some embodiments, the bacterium is a pathogenic bacterium. In some embodiments, the bacterium is an avirulent bacterium. In some embodiments, the bacterium belongs to the *Enterobaceteriaceae*. In some embodiments, the bacterium belongs to a genus selected from: *Alterococcus*, *Aquamonas*, *Aranicola*, *Arsenophonus*, *Brenneria*, *Budvicia*, *Buttiauxella*, *Candidatus Phlomobacter*, *Cedeceae*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Ewingella*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Leminorella*, *Moellerella*, *Morganella*, *Obesumbacterium*, *Pantoea*, *Pectobacterium*, *Photorhabdus*, *Plesiomonas*, *Pragia*, *Proteus*, *Providencia*, *Rahnella*, *Raoultella*, *Salmonella*, *Samsonia*, *Serratia*, *Shigella*, *Sodalis*, *Tatumella*, *Trabulsiella*, *Wigglesworthia*, *Xenorhbdus*, *Yersinia*, *Yokenella*. In some embodiments, the bacterium is a pathogenic species of *Enterobaceteriaceae*. In some embodiments, the bacterium is selected from the group consisting of *Escherichia coli*, *Shigella*, *Edwardsiella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia* and *Yersinia*. In some embodiments, the bacterium is of the genus *Salmonella.* In some embodiments, the bacterium is of the genus *Yersinia.* In some embodiments, the bacterium is of the genus *Edwardsiella*. In some embodiments, the bacterium is of a genus, species, or strain commonly used as a live or attenuated vaccine.

[0068] Some embodiments of the instant disclosure comprise a species or subspecies of the *Salmonella* genera (*e.g.*, *S. enterica* or *S. bongori*). For instance, the recombinant bacterium may be a *Salmonella enterica* serovar, including, for example, Paratyphi A, Enteritidis, Typhi, and Typhimurium. In some embodiments, the recombinant bacterium is of the serovar *S.* Typhimurium, *S.* Typhi, *S.* Paratyphi, *S.* Gallinarum, *S.* Enteritidis, *S.* Choleraesius, *S.* Arizonae, *S.* Newport, *S.* Heidelberg, *S.* Infantis, *S.* Cholerasiuis, or *S.* Dublin.

[0069] A recombinant bacterium derived from *Salmonella* may be particularly suited to use as a vaccine. For example, oral infection of a host with a *Salmonella* strain typically leads to colonization of the gut-associated lymphoid tissue (GALT), which leads to the induction of a generalized mucosal immune response to the recombinant bacterium. Further penetration of the bacterium into the mesenteric lymph nodes, liver and spleen may augment the induction of systemic and cellular immune responses directed against the bacterium.

Thus, the use of recombinant *Salmonella* for oral immunization stimulates all three branches of the immune system, which is particularly important for immunizing against infectious disease agents that colonize on and/or invade through mucosal surfaces. In some embodiments, the recombinant bacterium described herein is used to induce an immune response in poultry (*e.g.*, as a vaccine). When used in poultry, the recombinant bacterium may be administered by course spray and thereby inoculate the conjunctiva-associated lymphoid tissue (CALT) via eye exposure, the nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) via respiratory exposure and the GALT via oral exposure. In some embodiments, the recombinant bacterium described herein is administered to newly-hatched chicks.

A. Antigens

[0070] As used herein, "antigen" refers to a biomolecule capable of eliciting an immune response in a host. In some embodiments, an antigen may be a protein, or fragment of a protein. In some embodiments, the recombinant bacterium comprises a nucleic acid (*e.g.*, a plasmid) encoding an antigen of interest, wherein the nucleic acid is expressed by the host cell (*e.g.*, a DNA vaccine). In some embodiments, the recombinant bacterium comprises a nucleic acid encoding an antigen of interest. In some embodiments, the recombinant bacterium comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight or more nucleic acids encoding an antigen of interest (*e.g.*, one or more copies of a nucleic acid encoding a specific antigen, one or more nucleic acids encoding different antigens of interest, or combinations thereof). In an exemplary embodiment, the antigen elicits a protective immune response in a subject.

[0071] As used herein, "protective" means that the immune response contributes to the lessening of any symptoms associated with infection of a host with the pathogen the antigen was derived from or designed to elicit a response against. For example, a protective antigen from a pathogen, such as *Salmonella*, may induce an immune response that helps to ameliorate symptoms associated with *Salmonella* infection or reduce the morbidity and mortality associated with infection with the pathogen or may reduce the ability of *Salmonella* to infect and colonize the host. The use of the term "protective" in this disclosure does not necessarily require that the host is completely protected from the effects of the pathogen.

[0072] *C. jejuni* is a commensal for poultry and causes no disease symptoms. Thus, when "protective" immunity to *C. jejuni* is induced in poultry, this refers to the ability of the

vaccine to reduce the levels of Cj colonization in the intestine and ceca. This is also true for *Salmonella.* Thus, although several stains of *Salmonella* can cause disease and mortality in newly hatched chicks, chicks become totally tolerant of *Salmonella* by one week of age, after which most strains of *Salmonella* persist in the GI tract as a commensal.

[0073] In some embodiments, the antigen of interest is an antigen derived from an infectious agent. In some embodiments, the antigen of interest is derived from an infectious agent selected from the group consisting of a virus, a bacteria, a protozoan, a prion, a fungi, and a helminth. In some embodiments, the antigen of interest is derived from a bacteria.

[0074] Alternatively, antigens may be derived from organisms newly identified or newly associated with a disease or pathogenic condition, or new or emerging pathogens of animals or humans, including those now known or identified in the future, may be expressed by a bacterium detailed herein. Furthermore, antigens are not limited to those from pathogenic organisms.

[0075] Immunogenicity of the bacterium may be augmented and/or modulated by constructing strains that also express sequences for cytokines, adjuvants, and other immunomodulators.

[0076] Some examples of microorganisms useful as a source for antigen are listed below. These may include microorganisms for the control of plague caused by *Yersinia pestis* and other *Yersinia* species such as *Y. pseudotuberculosis* and *Y. enterocolitica*, for the control of gonorrhea caused by *Neisseria gonorrhoea*, for the control of syphilis caused by *Treponema pallidum*, and for the control of venereal diseases as well as eye infections caused by *Chlamydia trachomatis*. Species of *Streptococcus* from both group A and group B, such as those species that cause sore throat or heart diseases, *Streptococcus equi,* which causes strangles in equines, *Streptococcus mutans,* which causes cavities, and *Streptococcus pneumoniae, Erysipelothrix rhusiopathiae*, *Neisseria meningitidis*, *Mycoplasma pneumoniae* and other Mycoplasma-species, *Hemophilus influenza*, *Bordetella pertussis*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, other *Bordetella* species, *Escherichia coli*, *Streptococcus equi*, *Streptococcus pneumoniae*, *Brucella abortus*, *Pasteurella hemolytica* and *P. multocida*, *Vibrio cholera*, *Shigella* species, *Borrellia* species, *Bartonella* species, *Heliobacter pylori*, *Campylobacter* species, *Pseudomonas* species, *Moraxella* species, *Brucella* species, *Francisella* species, *Aeromonas* species, *Actinobacillus* species, *Clostridium* species, *Rickettsia* species, *Bacillus* species, *Coxiella* species, *Ehrlichia* species,

Listeria species, and *Legionella pneumophila* are additional examples of bacteria within the scope of this disclosure from which antigen nucleic acid sequences could be obtained.

[0077] In some embodiments, the antigen is a *Campylobacter* antigen (e.g., a *C. jejuni* antigen or a *C. coli* antigen). In some embodiments, the *Campylobacter* antigen is selected from the group consisting of Peb1 (encoded by the *cj0921c* gene), CjaA (encoded by the *cj0982c* gene), Dps (encoded by the *cj1534c* gene), TlyA (encoded by the *cj0588* gene), Omp18 (encoded by the *cj0113* gene), Cj0998c (encoded by the *cj0998c* gene), Cj0034c (encoded by the *cj0034c* gene), Cj0168c (encoded by the *cj0168c* gene), Cj0248 (encoded by the *cj0248* gene), Peb3 (encoded by the *cj0289* gene), CmeC (encoded by the *cj0365* gene), Cj0404 (encoded by the *cj0404* gene), Cj0420 (encoded by the *cj0420* gene), Cj0427 (encoded by the *cj0427* gene), Cj0428 (encoded by the *cj0428* gene), PorA (encoded by the *cj1259* gene), Fla (encoded by the *cj1339c* gene), CadF (encoded by the *cj1478c* gene), and Cj1656c (encoded by the *cj1656c* gene).

[0078] In some embodiments, the *Campylobacter* antigen comprises a canonical *Campylobacter* N-glycosylation amino acid sequence. In some embodiments, the canonical *Campylobacter* N-glycosylation amino acid sequence comprises the amino acid sequence Asp/Glu-Xaa-Asn-Tyr-Ser/Thr (SEQ ID NO: 1). The presence of the canonical *Campylobacter* N-glycosylation amino acid sequence allows for the glycosylation of the antigen when produced by a recombinant bacterium comprising a *pgl* operon (e.g., a *Campylobacter pgl* operon) or one or more *pgl* operon genes (*e.g.*, *wlaA, gne, pglK, pglH, pglI, pglJ, pglB, pglA, pglC, pglD, wlaJ, pglE, pglF,* and *pglG*). In one embodiment, the PglB enzyme can add the N-glycan to a slightly different aa sequence, DGGK (SEQ ID NO:2), different from the N-glycosylation sequence used in eukaryotes (Barre et al., 2017. Glycobiology 27:978-989).

[0079] In some embodiments, the antigen of interest comprises a native canonical *Campylobacter* N-glycosylation amino acid sequence. In some embodiments, the antigen of interest is engineered to comprise at least one non-native canonical *Campylobacter* N-glycosylation amino acid sequence Asp/Glu-Xaa-Asn-Tyr-Ser/Thr (SEQ ID NO: 1), such that the antigen is N-glycosylated when produced by a recombinant bacterium comprising a *pgl* operon (*e.g.*, a *Campylobacter pgl* operon) or one or more *pgl* operon genes.

[0080] In certain embodiments, an antigen may comprise a B-cell epitope or a T-cell epitope. Alternatively, an antigen to which an immune response is desired may be expressed as a fusion to a carrier protein that contains a strong promiscuous T-cell epitope and/or serves

as an adjuvant and/or facilitates presentation of the antigen to enhance, in all cases, the immune response to the antigen or its component part. This can be accomplished by methods known in the art. Fusion to tetanus toxin fragment C, CT-B, LT-B and hepatitis virus B core are particularly useful for these purposes, although other epitope presentation systems are well known in the art.

[0081] In further embodiments, a nucleic acid sequence encoding an antigen may comprise a secretion signal.

[0082] As stated above, the level of synthesis of an antigen of interest may be optimized by modifying the nucleic acid sequence encoding the repressor and/or promoter. As used herein, "modify" refers to an alteration of the nucleic acid sequence of the repressor and/or promoter that results in a change in the level of transcription of the nucleic acid sequence encoding the repressor, or that results in a change in the level of synthesis of the repressor. For instance, in one embodiment, modify may refer to altering the start codon of the nucleic acid sequence encoding the repressor. Generally speaking, a GTG or TTG start codon, as opposed to an ATG start codon, may decrease translation efficiency ten-fold. In another embodiment, modify may refer to altering the Shine-Dalgarno (SD) sequence of the nucleic acid sequence encoding the repressor. The SD sequence is a ribosomal binding site generally located 6-7 nucleotides upstream of the start codon. The SD consensus sequence is AGGAGG (SEQ ID NO:80), and variations of the consensus sequence may alter translation efficiency. In yet another embodiment, modify may refer to altering the distance between the SD sequence and the start codon. In still another embodiment, modify may refer to altering the –35 sequence for RNA polymerase recognition. In a similar embodiment, modify may refer to altering the –10 sequence for RNA polymerase binding. In an additional embodiment, modify may refer to altering the number of nucleotides between the –35 and –10 sequences. In an alternative embodiment, modify may refer to optimizing the codons of the nucleic acid sequence encoding the repressor to alter the level of translation of the mRNA encoding the repressor. For instance, non-A rich codons initially after the start codon of the nucleic acid sequence encoding the repressor may not maximize translation of the mRNA encoding the repressor. Similarly, the codons of the nucleic acid sequence encoding any of the proteins described herein may be codon-optimized, *i.e.*, altered so as to mimic the codons from highly synthesized proteins of a particular organism. In a further embodiment, modify may refer to altering the GC content of the nucleic acid sequence encoding the repressor to

change the level of translation of the mRNA encoding the repressor. Methods of modifying a nucleic acid sequence are known in the art.

[0083] In some embodiments, more than one modification or type of modification may be performed to optimize the expression level of a nucleic acid described herein (*e.g.*, a nucleic acid encoding a repressor or antigen of interest). For instance, at least one, two, three, four, five, six, seven, eight, or nine modifications, or types of modifications, may be performed to optimize the expression level of a nucleic acid described herein. By way of non-limiting example, when the repressor is LacI, then the nucleic acid sequence of LacI and the promoter may be altered so as to increase the level of LacI synthesis. In one embodiment, the start codon of the LacI repressor may be altered from GTG to ATG. In another embodiment, the SD sequence may be altered from AGGG to AGGA. In yet another embodiment, the codons of *lacI* may be optimized according to the codon usage for highly synthesized proteins of *Salmonella*. In a further embodiment, the start codon of *lacI* may be altered, the SD sequence may be altered, and the codons of *lacI* may be optimized.

[0084] In some embodiments, the recombinant bacterium comprises a nucleic acid that is located in a plasmid or vector. As used herein, "vector" refers to an autonomously replicating nucleic acid unit. The present disclosure can be practiced with any known type of vector, including viral, cosmid, phasmid, and plasmid vectors. The most preferred type of vector is a plasmid vector. In some embodiments, the plasmid or vector is a high copy plasmid. In some embodiments, the plasmid or vector is a low copy plasmid or vector.

[0085] As is well known in the art, plasmids and other vectors may possess a wide array of promoters, multiple cloning sequences, transcription terminators, etc., and vectors may be selected so as to control the level of expression of the nucleic acid sequence encoding an antigen by controlling the relative copy number of the vector. In some instances in which the vector might encode a surface localized adhesin as the antigen, or an antigen capable of stimulating T-cell immunity, it may be preferable to use a vector with a low copy number such as at least two, three, four, five, six, seven, eight, nine, or ten copies per bacterial cell. A non-limiting example of a low copy number vector may be a vector comprising the pSC101 *ori*.

[0086] In some embodiments, the plasmid comprises a nucleic acid sequence encoding an aspartate-semialdehyde dehydrogenase gene (*e.g.*, *asdA*). These plasmids may be advantageously used to complement a bacterium that comprises an aspartate-semialdehyde

dehydrogenase gene (*e.g.*, *asdA*). In some embodiments, the plasmid is selected from the group consisting of pYA3342, pYA3337, and pYA3332.

[0087] In other cases, an intermediate copy number vector might be optimal for inducing desired immune responses. For instance, an intermediate copy number vector may have at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 copies per bacterial cell. A non-limiting example of an intermediate copy number vector may be a vector comprising the p15A *ori*.

[0088] In still other cases, a high copy number vector might be optimal for the induction of maximal antibody responses. A high copy number vector may have at least 31, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 copies per bacterial cell. In some embodiments, a high copy number vector may have at least 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 400 copies per bacterial cell. Non-limiting examples of high copy number vectors may include a vector comprising the pBR ori or the pUC *ori*.

[0089] Additionally, vector copy number may be increased by selecting for mutations that increase plasmid copy number. These mutations may occur in the bacterial chromosome but are more likely to occur in the plasmid vector.

[0090] Preferably, vectors used herein do not comprise antibiotic resistance markers to select for maintenance of the vector.

[0091] In some embodiments, the nucleic acid sequences described herein are operably-linked to a promoter. Promoters for use in the embodiments described herein are known in the art. One of skill in the art would recognize that the selection of a repressor dictates, in part, the selection of the promoter to be used to regulate the expression of a nucleic acid described herein. For instance, if the repressor is LacI, then the promoter may be selected from the group consisting of LacI responsive promoters, such as P_{trc}, P_{lac}, P_{T7lac} and P_{tac}. If the repressor is C2, then the promoter may be selected from the group consisting of C2 responsive promoters, such as P22 promoters P_L and P_R. If the repressor is C1, then the promoter may be selected from the group consisting of C1 responsive promoters, such as λ promoters P_L and P_R.

[0092] In each embodiment herein, the promoter regulates expression of a nucleic acid sequence. In some embodiments, the promoter comprises a regulatory sequence controlled by a repressor, such that expression of the nucleic acid sequence is repressed when the repressor is synthesized (*e.g.*, during *in vitro* growth of the bacterium), but expression of the nucleic acid sequence encoding an antigen is high when the repressor is not synthesized

(*e.g.*, *in vivo*). Generally speaking, the concentration of the repressor will decrease with every cell division after expression of the gene encoding the repressor ceases. In some embodiments, the concentration of the repressor decreases such that high levels of expression of the nucleic acid sequence that is being regulated is achieved after about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 divisions of the bacterium. In an exemplary embodiment, the concentration of the repressor decreases enough to allow high-level expression of the nucleic acid sequence encoding an antigen after about 5 divisions of the bacterium *in vivo*.

[0093] In certain embodiments, the promoter may comprise other regulatory elements. For instance, the promoter may comprise *lacO* if the repressor is LacI. This is the case with the lipoprotein promoter P_{lpp}*lacO* that is regulated by LacI since it possesses the LacI binding domain *lacO*. In one embodiment, the repressor is a LacI repressor and the promoter is P_{trc}.

[0094] In some embodiments, the expression of the nucleic acid sequence regulated by a repressor is repressed *in vivo*. Expression may be "repressed" or "partially repressed" when it is about 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, or even less than 1% of the expression under non-repressed conditions. Thus although the level of expression under conditions of "complete repression" might be exceeding low, it is likely to be detectable using very sensitive methods since repression can never by absolute.

[0095] Conversely, the expression of the nucleic acid sequence encoding the antigen should be high when the expression of the repressor is repressed. For instance, if the repressor is not expressed during growth of the recombinant bacterium in a host, the expression of the nucleic acid under the control of the repressor will be high. As used herein, "high level" expression refers to expression that is strong enough to elicit an immune response to the antigen. Consequently, the copy number correlating with high level expression can and will vary depending on the antigen and the type of immune response desired. Methods of determining whether an antigen elicits an immune response such as by measuring antibody levels or antigen-dependent T cell populations or antigen-dependent cytokine levels are known in the art, and methods of measuring levels of expression of antigen encoding sequences by measuring levels of mRNA transcribed or by quantitating the expression level of a protein are also known in the art.

[0096] In each of the above embodiments, a recombinant bacterium capable of regulated expression may also be attenuated. "Attenuated" refers to the state of the bacterium wherein the bacterium has been weakened from its wild-type fitness by some form of

recombinant or physical manipulation. This includes altering the genotype of the bacterium to reduce its ability to cause disease. However, the bacterium's ability to colonize the gut (in the case of *Salmonella*) and induce immune responses is, preferably, not substantially compromised.

[0097] In an exemplary embodiment, a recombinant bacterium may be attenuated as described above. In which case, both regulated attenuation and regulated expression of an antigen encoding sequence may be dependent upon a sugar regulatable system. Consequently, the concentration of sugar (*e.g.*, arabinose) needed for optimal expression of the regulated antigen encoding sequence may not be the same as the concentration for optimal expression of attenuation. In an exemplary embodiment, the concentration of arabinose for the optimization of both regulated attenuation and regulated expression of sequences encoding antigen will be substantially the same.

[0098] Accordingly, the promoter and/or the nucleic acid sequence encoding an attenuation protein may be modified to optimize the system. Methods of modification are detailed above. Briefly, for example, the SD ribosome binding sequence may be altered, and/or the start codon may be altered from ATG to GTG for the nucleic acid sequences *fur* and *phoPQ*, so that the production levels of Fur and PhoPQ are optimal for both the regulated attenuation phenotype and the regulated expression when growing strains with a given concentration of arabinose. One of skill in the art will appreciate that other nucleic acid sequences, in addition to *fur* and *phoPQ*, may also be altered as described herein in combination with other well-known protocols. In addition, these attenuating nucleic acid sequences may be regulated by other systems using well-established protocols known to one of skill in the art. For example, they may be regulated using with promoters dependent on addition of maltose, rhamnose, or xylose rather than arabinose.

[0099] *cj0034c* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0034c* gene (e.g., a *C. jejuni cj0034c* gene).

[00100] In some embodiments, the nucleic acid comprising a *cj0034c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0034c* gene is located on a chromosome of the bacterium.

[00101] The nucleic acid sequence of an exemplary *C. jejuni cj0034c* gene is provided below:

ATGAAAACAAATAATATCTTTATGGCTTTAGCCATAGTTTTGGCAAGTTTGATTCTAGCTTT
TGGATTTAACAAGGCTTTAAGTGATTTTAAAACACTTGAAAGAAGTGTAAGTGTAAAGGGTT

TAAGTCAAAAAGAAGTCGAAGCGGATACTTTGATACTTCCTATAAAATTCACAAGATCAAAC
AACAATCTTACAAATTTATACGAAGAACTAGAACAAGATAAAGAAAATATCATCAAATTTTT
AGAAAAACAAGGCATAAAAGAAGATGAGATCAGCTACAACTCGCCAAATATCATAGATCGTT
TAAGCGATCCTTATAGCAACGACACTCAAGCTGCATACCGATACATAGGCACTGCGAATTTA
CTCATCTATACTCAAAATGTAAAGCTTGGAAAAAGCATACTAGAAAACATTTCAAGTCTTGC
AAAATTTGGTATAGTAACAAAAATCGATGATTATGATATAGAATACCTTTACACCAAGCTAA
ATGATATAAAACCACAAATGATAGAAGAAGCAACGCTCAATGCTAGAAATGCAGCGATAAAA
TTCGCACAAGACTCAAACAGCCATCTAGGCAAGATAAAAAAGGCTTCTCAAGGACAATTTAG
CATTAGCAACAGAGATAAAAACACCCCTTATATCAAAACCATAAGAGTGGTTTCTACTATAG
AATACTACTTAAAAGACTGA (SEQ ID NO: 3)

[00102] The amino acid sequence of the Cj0034c protein encoded by the nucleic acid of SEQ ID NO: 3 is provided below:

MKTNNIFMALAIVLASLILAFGFNKALSDFKTLERSVSVKGLSQKEVEADTLILPIKFTRSN
NNLTNLYEELEQDKENIIKFLEKQGIKEDEISYNSPNIIDRLSDPYSNDTQAAYRYIGTANL
LIYTQNVKLGKSILENISSLAKFGIVTKIDDYDIEYLYTKLNDIKPQMIEEATLNARNAAIK
FAQDSNSHLGKIKKASQGQFSISNRDKNTPYIKTIRVVSTIEYYLKD (SEQ ID NO: 4)

[00103] In some embodiments, the nucleic acid comprises a *C. jejuni cj0034c* gene (provided as SEQ ID NO: 3). In some embodiments, the nucleic acid comprises a *cj0034c* gene, wherein the *cj0034c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 3. In some embodiments, the nucleic acid comprises a *cj0034c* gene, wherein the *cj0034c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 3.

[00104] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0034c protein, wherein said Cj0034c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 4. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0034c

protein, wherein said Cj0034c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 4.

[00105] *cj0113* –In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0113* gene (*e.g.*, a *C. jejuni cj0113* gene).

[00106] In some embodiments, the nucleic acid comprising a *cj0113* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0113* gene is located on a chromosome of the bacterium.

[00107] The nucleic acid sequence of an exemplary *C. jejuni cj0113* gene is provided below:

ATGAAAAAAGTTTTATTGAGTTCATTGGTTGCGGTGTCTTTGTTAAGCACAGGTTTGTTTGC
TAAAGAATATACTTTAGATAAAGCACATACAGATGTAGGTTTTAAAATCAAACATTTACAAA
TTAGCAATGTAAAAGGAAATTTCAAAGATTATTCTGCGGTGATTGATTTTGATCCTGCGAGT
GCTGAATTTAAAAAGCTTGATGTAACTATAAAAATCGCATCTGTAAATACAGAAAATCAAAC
AAGAGATAATCACTTACAACAAGATGATTTTTTCAAAGCAAAAAAATATCCTGATATGACTT
TTACAATGAAAAAATATGAAAAAATCGATAATGAAAAAGGCAAAATGACAGGAACTTTAACT
ATAGCTGGAGTTTCTAAAGATATCGTTTTAGATGCTGAAATCGGCGGTGTAGCTAAAGGCAA
AGATGGAAAAGAAAAAATAGGATTTTCTTTAAATGGAAAAATCAAACGCTCTGATTTTAAAT
TTGCAACAAGTACTTCAACTATTACTTTAAGTGATGATATTAATTTAAATATCGAAGTTGAA
GCGAACGAAAAATAA (SEQ ID NO: 5)

[00108] The amino acid sequence of the Omp18 protein encoded by the nucleic acid of SEQ ID NO: 5 is provided below:

MKKVLLSSLVAVSLLSTGLFAKEYTLDKAHTDVGFKIKHLQISNVKGNFKDYSAVIDFDPASAE
FKKLDVTIKIASVNTENQTRDNHLQQDDFFKAKKYPDMTFTMKKYEKIDNEKGKMTGTLTIA
GVSKDIVLDAEIGGVAKGKDGKEKIGFSLNGKIKRSDFKFATSTSTITLSDDINLNIEVEANEK
(SEQ ID NO: 6)

[00109] In some embodiments, the nucleic acid comprises a *C. jejuni cj0113* gene (provided as SEQ ID NO: 5). In some embodiments, the nucleic acid comprises a *cj0113* gene, wherein the *cj0113* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

identical to the nucleic acid sequence of SEQ ID NO: 5. In some embodiments, the nucleic acid comprises a *cj0113* gene, wherein the *cj0113* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 5.

[00110] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Omp18 protein, wherein said Omp18 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Omp18 protein, wherein said Omp18 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 6.

[00111] *cj0168c* – In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0168c* gene (*e.g.*, a *C. jejuni cj0168c* gene).

[00112] In some embodiments, the nucleic acid comprising a *cj0168c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0168c* gene is located on a chromosome of the bacterium.

[00113] The nucleic acid sequence of an exemplary *C. jejuni cj0168c* gene is provided below:

ATGAAAAAAGTTGTACTAATCTCAGCATTACTAGGTGCTTTCGCAGCTAATGTTTTTGCAGC
TAATACTCCAAGCGATGTAAATCAAACACATACAAAAGCTAAAGCTGATAAAAAACATGAAG
CTAAAACTCACAAAAAAACAAAAGAGCAAACACCAGCTCAATAA (SEQ ID NO: 7)

[00114] The amino acid sequence of the Cj0168c protein encoded by the nucleic acid of SEQ ID NO: 7 is provided below:

MKKVVLISALLGAFAANVFAANTPSDVNQTHTKAKADKKHEAKTHKKTKEQTPAQ (SEQ ID NO: 8)

[00115] In some embodiments, the nucleic acid comprises a *C. jejuni cj0168c* gene (provided as SEQ ID NO: 7). In some embodiments, the nucleic acid comprises a *cj0168c* gene, wherein the *cj0168c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 7. In some embodiments, the nucleic acid comprises a *cj0168c* gene, wherein the *cj0168c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 7.

[00116] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0168c protein, wherein said Cj0168c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 8. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0168c protein, wherein said Cj0168c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 8.

[00117] *cj0248* – In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0248* gene (*e.g.*, a *C. jejuni cj0248* gene).

[00118] In some embodiments, the nucleic acid comprising a *cj0248* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0248* gene is located on a chromosome of the bacterium.

[00119] The nucleic acid sequence of an exemplary *C. jejuni cj0248* gene is provided below:

ATGATTGGAGATATGAATGAGCTTTTATTAAAAAGCGTTGAAGTATTGCCACCTTTACCTGA
TACTGTAAGTAAGTTAAGAAAATATGTGAGCGAGGCTAATTCAAATATAGAAACTATGAAAG

TTGCTGAAATCATTTCAAGCGATCCGTTGATGACGGCTAAGCTTTTGCAATTAGCAAATTCT
CCTTATTATGGTTTTACAAGAGAAATTACAACCATAAATCAAGTGATTACTTTATTAGGCGT
TGGTAATATCATCAATATAGTTATGGCTGACTCCATTAGAGATAATTTTAAAATAGACGTTT
CACCTTATGGTTTAAATACTCAAAATTTTTTAAAAACGTGCAATGAAGAGGCAACTTTTATC
GCAAATTGGCTTAATGATGAAGATAAAAAACTTTCTCATCTTTTAGTTCCTTGTGCAATGCT
TTTAAGGCTTGGTATTGTTATTTTTTCAAATTTTCTTATACAAAATCATAAGGATAAGGATT
TTTTAGCTTTTTTAAATAAAAATGAAAATCTTGCTTTAGCGGAGAATGAATTTTTAGGCGTA
GATCATATTTCTTTCTTGGGATTTTTGTTACATCGTTGGAATTTTGATGATGTTTTGATTGA
AAGTATATGTTTTGTTCGCACTCCTCATGCTGCTCGCGAAAAAGTGAAAAAATCCGCTTATG
CTTTAGCAATAACAGATCATCTTTTTGCTCCGCATGATGGTTCTTCTCCATTTAACGCAAAA
GCTGCAGTTGCTTTACTTAAAGAGGCAAAAACTCAAGGAATTAATTTTGATTTAAACAATCT
TTTATCTAAGCTTCCTAACAAAGCTAAGGAAAATTTAAACAAAGAAGATTAA (SEQ ID NO: 9)

[00120] The amino acid sequence of the Cj0248 protein encoded by the nucleic acid of SEQ ID NO: 9 is provided below:

MIGDMNELLLKSVEVLPPLPDTVSKLRKYVSEANSNIETMKVAEIISSDPLMTAKLLQLANSPY
YGFTREITTINQVITLLGVGNIINIVMADSIRDNFKIDVSPYGLNTQNFLKTCNEEATFIANWLN
DEDKKLSHLLVPCAMLLRLGIVIFSNFLIQNHKDKDFLAFLNKNENLALAENEFLGVDHISFLG
FLLHRWNFDDVLIESICFVRTPHAAREKVKKSAYALAITDHLFAPHDGSSPFNAKAAVALLKE
AKTQGINFDLNNLLSKLPNKAKENLNKED (SEQ ID NO: 10)

[00121] In some embodiments, the nucleic acid comprises a *C. jejuni cj0248* gene (provided as SEQ ID NO: 9). In some embodiments, the nucleic acid comprises a *cj0248* gene, wherein the *cj0248* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 9. In some embodiments, the nucleic acid comprises a *cj0248* gene, wherein the *cj0248* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 9.

[00122] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0248 protein, wherein said Cj0248 protein comprises an amino acid

sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 10. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0248 protein, wherein said Cj0248 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 10.

[00123] *cj0289c* – In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0289c* gene (*e.g.*, a *C. jejuni cj0289c* gene).

[00124] In some embodiments, the nucleic acid comprising a *cj0289c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0289c* gene is located on a chromosome of the bacterium.

[00125] The nucleic acid sequence of an exemplary *C. jejuni cj0289c* gene is provided below:

ATGAAAAAAATTATTACTTTATTTGGTGCATGTGCCTTAGCTTTTAGTATGGCAA
ATGCAGACGTGAACCTGTACGGCCCGGGCGGCCCGCACACGGCCCTGAAAGACA
TCGCAAACAAATATAGCGAAAAAACCGGCGTGAAAGTGAACGTGAACTTTGGCC
CGCAGGCGACCTGGTTTGAAAAAGCGAAAAAAGACGCGGACATCCTGTTTGGCG
CGTCAGACCAGTCCGCTCTGGCTATCGCGAGCGACTTTGGCAAAGACTTTAACGT
GAGCAAAATCAAACCGCTGTATTTTCGTGAAGCCATCATCCTGACCCAGAAAGG
CAACCCGCTGAAAATCAAAGGCCTGAAAGACCTGGCGAACAAAAAAGTGCGTAT
CGTGGTGCCGGAAGGCGCGGGCAAAAGCAACACCTCTGGCACCGGCGTGTGGGA
AGACATGATCGGCCGTACCCAGGACATCAAAACCATCCAGAACTTTCGTAACAA
CATCGTGGCCTTTGTGCCGAACAGCGGTAGCGCGCGTAAACTGTTCGCGCAGGAC
CAGGCCGACGCTTGGATCACTTGGATCGACTGGTCAAAAAGCAACCCGGACATC
GGCACTGCCGTGGCTATCGAAAAAGACCTGGTGGTGTATCGTACTTTTAACGTGA
TCGCGAAAGAAGGCGCGAGCAAAGAAACACAGGACTTTATCGCTTATCTGAGTT
CTAAAGAAGCGAAAGAAATCTTTAAAAAATACGGCTGGCGTGAATAA (SEQ ID NO: 11)

[00126] The amino acid sequence of the Peb3 protein encoded by the nucleic acid of SEQ ID NO: 11 is provided below:
MKKIITLFGACALAFSMANADVNLYGPGGPHTALKDIANKYSEKTGVKVNVNFGPQ ATWFEKAKKDADILFGASDQSALAIASDFGKDFNVSKIKPLYFREAIILTQKGNPLKIK GLKDLANKKVRIVVPEGAGKSNTSGTGVWEDMIGRTQDIKTIQNFRNNIVAFVPNSG SARKLFAQDQADAWITWIDWSKSNPDIGTAVAIEKDLVVYRTFNVIAKEGASKETQD FIAYLSSKEAKEIFKKYGWRE (SEQ ID NO: 12)

[00127] In some embodiments, the nucleic acid comprises a *C. jejuni cj0289c* gene (provided as SEQ ID NO: 11). In some embodiments, the nucleic acid comprises a *cj0289c* gene, wherein the *cj0289c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 11. In some embodiments, the nucleic acid comprises a *cj0289c* gene, wherein the *cj0289c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 11.

[00128] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Peb3 protein, wherein said Peb3 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Peb3 protein, wherein said Peb3 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 12.

[00129] *cj0365c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0365c* gene (*e.g.*, a *C. jejuni cj0365c* gene).

[00130] In some embodiments, the nucleic acid comprising a *cj0365c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0365c* gene is located on a chromosome of the bacterium.

[00131] The nucleic acid sequence of an exemplary *C. jejuni cj0365c* gene is provided below:

ATGAATAAAATAATTTCAATTAGTGCTATAGCAAGTTTTACTCTTTTGATTTCAGCTTGCTC
TTTAAGTCCAAATTTAAATATTCCCGAAGCAAACTATAGCATTGATAATAAGCTTGGAGCCT
TATCTTGGGAAAAAGAAAACAATAGCTCTATCACAAAAAATTGGTGGAAAGACTTTGATGAT
GAAAATTTAAATAAAGTGGTTGATTTAGCACTTAAAAATAATAATGATTTAAAACTTGCTTT
CATACACATGGAACAAGCTGCTGCTCAATTAGGTATAGATTTTAGCAGTTTGTTGCCAAAAT
TTGATGGTAGCGCAAGCGGAAGTCGTGCAAAAACAGCTATAAATGCTCCAAGCAATCGAACT
GGGGAAGTAAGTTACGGTAATGATTTTAAAATGGGACTTAATTTAAGCTATGAAATCGATCT
TTGGGGAAAATATCGCGATACATATCGCGCCTCAAAATCAGGCTTTAAAGCAAGTGAGTATG
ATTATGAAGCTGCAAGACTTTCTGTTATTTCAAATACAGTTCAAACTTATTTTAATCTTGTA
AATGCTTATGAAAATGAAAATGCTCTTAAAGAAGCCTATAAATCTGCAAAAGAAATTTATAG
GATTAATGATGAAAAATTTCAAGTTGGTGCTGTAGGTGAATATGAACTTGCTCAAGCAAGAG
CCAACTTAGAAAGTATGGCTTTGCAATATAATGAAGCAAAGTTAAATAAAGAAAATTACCTT
AAAGCTTTAAAAATTTTAACTTCAAATGATTTAAATGACATACTTTACAAAAATCAAAGCTA
TCAAGTTTTTAATCTTAAAGAATTTGACATTCCAACTGGAATTTCAAGTACCATCTTGCTTC
AACGTCCAGATATTGGCTCTTCTTTAGAAAAATTAACTCAGCAAAATTATCTTGTTGGAGTA
GCTCGCACGGCTTTCTTACCTAGCCTTTCTTTAACAGGATTATTGGGATTTGAAAGCGGGGA
TTTAGATACCTTGGTTAAAGGAGGTTCTAAGACTTGGAATATAGGTGGAAACTTTACTCTGC
CTATTTTTCATTGGGGTGAAATTTACCAAAATGTAAATTTAGCCAAGCTTAATAAAGATGAA
GCTTTTGTAAATTATCAAAATACTTTGATTACTGCTTTTGGAGAAATTCGCTATGCTTTAGT
AGCTAGAAAAACTATACGCTTACAATACGATAATGCACAAGCAAGCGAACAATCTTACAAAA
GAATCTATGAAATTGCTAAAGAACGCTATGATATAGGAGAAATGTCTTTGCAAGATTATTTA
GAGGCACGTCAAAATTGGCTTAATGCTGCGGTTGCTTTTAATAATATTAAATATTCTTATGC
CAATTCCATAGTAGATGTAATCAAAGCATTTGGTGGAGGATTTGAGCAAAGTGAAGATACGA
GTAAAAATATAAAAGAAGAATCAAAAAATTTAGATATGTCTTTTAGAGAATAG (SEQ ID NO: 13)

[00132] The amino acid sequence of the CmeC protein encoded by the nucleic acid of SEQ ID NO: 13 is provided below:

MNKIISISAIASFTLLISACSLSPNLNIPEANYSIDNKLGALSWEKENNSSITKNWWKDFDDENL
NKVVDLALKNNNDLKLAFIHMEQAAAQLGIDFSSLLPKFDGSASGSRAKTAINAPSNRTGEVS
YGNDFKMGLNLSYEIDLWGKYRDTYRASKSGFKASEYDYEAARLSVISNTVQTYFNLVNAYEN
ENALKEAYKSAKEIYRINDEKFQVGAVGEYELAQARANLESMALQYNEAKLNKENYLKALKIL

TSNDLNDILYKNQSYQVFNLKEFDIPTGISSTILLQRPDIGSSLEKLTQQNYLVGVARTAFLPSLS LTGLLGFESGDLDTLVKGGSKTWNIGGNFTLPIFHWGEIYQNVNLAKLNKDEAFVNYQNTLIT AFGEIRYALVARKTIRLQYDNAQASEQSYKRIYEIAKERYDIGEMSLQDYLEARQNWLNAAVA FNNIKYSYANSIVDVIKAFGGGFEQSEDTSKNIKEESKNLDMSFRE (SEQ ID NO: 14)

[00133] In some embodiments, the nucleic acid comprises a *C. jejuni cj0365c* gene (provided as SEQ ID NO: 13). In some embodiments, the nucleic acid comprises a *cj0365c* gene, wherein the *cj0365c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 13. In some embodiments, the nucleic acid comprises a *cj0365c* gene, wherein the *cj0365c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 13.

[00134] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CmeC protein, wherein said CmeC protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 14. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CmeC protein, wherein said CmeC protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 14.

[00135] *cj0404* – In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0404* gene (*e.g.*, a *C. jejuni cj0404* gene).

[00136] In some embodiments, the nucleic acid comprising a *cj0404* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0404* gene is located on a chromosome of the bacterium.

[00137] The nucleic acid sequence of an exemplary *C. jejuni cj0404* gene is provided below:

ATGGAAAATCAAAAAAATGAATTTGATGATATTATTTTAGAAAAAAGTAATAAAAGTGAAAA
AGTAAAAAAAAATTCTTTTACGAGTTATTGCTTTAGTTATTTTGTTTTTAGCTATCATGATAG
TTATGAAGCTTATTAATGGTAGTGGTGATGAAAATACGCAAAATCAAAGTGTATTGCCAAGT
GAACCTATAGCAACTCAAGACAATAACAATGATACTTCTTTTGAAAGTATGCCAATTACAGA
TAATACTTCAGCAGAAGATCAATTTGAGGCATTAAGAAAACAATTTCAAGATGAACAAAATA
CAACTCAAAATACAACAACCTCTAGTTCAAATAACAATGATACTACAAATTTTGCTATGCCT
GATCAAGAAGTTCCAGCAGAACCAACAGCAACTACTTCAGCAAATACCACTCCACAAGCAAG
TACTCCTAAACAAGAAGTAACACAAACTGCAAAATCTAAAGAAGAAGCAAAAAAACAAACAG
CTGTAAAAAAAGAAAAAGAAAGTGCAAAACAAACCCCTAAAAAAGAACAAAATGCAAATGAT
TTATTTAAAAATGTTGATGCTAAACCTGTACATCCAAGTGGTTTAGCATCGGGTATTTATGT
GCAAATTTTCTCAGTAAGTAATTTGGATCAAAAATCAAAAGAACTTGCTTCTGTAAAGCAAA
AAGGTTATGATTATAAACTTTATAAAACTACAGTTGGAAGTAAAGAAATTACCAAGGTTTTA
ATAGGACCATTTGAAAAGGCAGATATTGCAGCAGAACTTGCTAAAATCCGTAAGGATATTGC
AAAAGATGCTTTTTCTTTTACTTTAAAATGA (SEQ ID NO: 15)

[00138] The amino acid sequence of the Cj0404 protein encoded by the nucleic acid of SEQ ID NO: 15 is provided below:

MENQKNEFDDIILEKSNKSEKVKKILLRVIALVILFLAIMIVMKLINGSGDENTQNQSVLPSEPIA
TQDNNNDTSFESMPITDNTSAEDQFEALRKQFQDEQNTTQNTTTSSSNNNDTTNFAMPDQE
VPAEPTATTSANTTPQASTPKQEVTQTAKSKEEAKKQTAVKKEKESAKQTPKKEQNANDLFK
NVDAKPVHPSGLASGIYVQIFSVSNLDQKSKELASVKQKGYDYKLYKTTVGSKEITKVLIGPFEK
ADIAAELAKIRKDIAKDAFSFTLK (SEQ ID NO: 16)

[00139] In some embodiments, the nucleic acid comprises a *C. jejuni cj0404* gene (provided as SEQ ID NO: 15). In some embodiments, the nucleic acid comprises a *cj0404* gene, wherein the *cj0404* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 15. In some embodiments, the nucleic acid comprises a *cj0404* gene, wherein the *cj0404* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 15.

[00140] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0404 protein, wherein said Cj0404 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0404 protein, wherein said Cj0404 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 16.

[00141] *cj0420*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0420* gene (*e.g.*, a *C. jejuni cj0420* gene).

[00142] In some embodiments, the nucleic acid comprising a *cj0420* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0420* gene is located on a chromosome of the bacterium.

[00143] The nucleic acid sequence of an exemplary *C. jejuni cj0420* gene is provided below:

ATGAAAAAAGTTTTATTGAGTTCATTGGTTGCGGTGTCTTTGTTAAGCACAGGTTTGTTTGC
TAAAGAATATACTTTAGATAAAGCACATACAGATGTAGGTTTTAAAATCAAACATTTACAAA
TTAGCAATGTAAAAGGAAATTTCAAAGATTATTCTGCGGTGATTGATTTTGATCCTGCGAGT
GCTGAATTTAAAAAGCTTGATGTAACTATAAAAATCGCATCTGTAAATACAGAAAATCAAAC
AAGAGATAATCACTTACAACAAGATGATTTTTTCAAAGCAAAAAAATATCCTGATATGACTT
TTACAATGAAAAAATATGAAAAAATCGATAATGAAAAAGGCAAAATGACAGGAACTTTAACT
ATAGCTGGAGTTTCTAAAGATATCGTTTTAGATGCTGAAATCGGCGGTGTAGCTAAAGGCAA
AGATGGAAAAGAAAAAATAGGATTTTCTTTAAATGGAAAAATCAAACGCTCTGATTTTAAAT
TTGCAACAAGTACTTCAACTATTACTTTAAGTGATGATATTAATTTAAATATCGAAGTTGAA
GCGAACGAAAAATAA (SEQ ID NO: 17)

[00144] The amino acid sequence of the Cj0420 protein encoded by the nucleic acid of SEQ ID NO: 17 is provided below:

MKKVLLSSLVAVSLLSTGLFAKEYTLDKAHTDVGFKIKHLQISNVKGNFKDYSAVIDFDPASAE
FKKLDVTIKIASVNTENQTRDNHLQQDDFFKAKKYPDMTFTMKKYEKIDNEKGKMTGTLTIA

GVSKDIVLDAEIGGVAKGKDGKEKIGFSLNGKIKRSDFKFATSTSTITLSDDINLNIEVEANEK (SEQ ID NO: 18)

[00145] In some embodiments, the nucleic acid comprises a *C. jejuni cj0420* gene (provided as SEQ ID NO: 17). In some embodiments, the nucleic acid comprises a *cj0420* gene, wherein the *cj0420* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 17. In some embodiments, the nucleic acid comprises a *cj0420* gene, wherein the *cj0420* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 17.

[00146] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0420 protein, wherein said Cj0420 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 18. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0420 protein, wherein said Cj0420 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 18.

[00147] *cj0427*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0427* gene (*e.g.*, a *C. jejuni cj0427* gene).

[00148] In some embodiments, the nucleic acid comprising a *cj0427* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0427* gene is located on a chromosome of the bacterium.

[00149] The nucleic acid sequence of an exemplary *C. jejuni cj0427* gene is provided below:

ATGATGGCTAAATTTAGAATTCAATACAGCGCAGGTTTTGGGCACTATACGCAAAATCACAA
GGGTTTTGGACCTACGATTTATATAGAAGAGGTCGTAGAGTTTGATAATGGCAAGGATTATT
TTGACTATATAGATTTTTATAAAACTTATTCAAAGAGCGATGATACTTATTTTCATATCAGT
TTTTTAGAAGATAGACCTCTAAGCGATAAAGAAATCACCATTCGCAATGAATACCGCAAAAT
GCGTGATGAAAACTGTAAAAAAGCCAAGGAGGAATTTATAGCCAACAATGAGCTTGATGTGG
AGCATTTGCCTACTCACCATGATTAA (SEQ ID NO: 19)

[00150] The amino acid sequence of the Cj0427 protein encoded by the nucleic acid of SEQ ID NO: 19 is provided below:

MMAKFRIQYSAGFGHYTQNHKGFGPTIYIEEVVEFDNGKDYFDYIDFYKTYSKSDDTYFHIS
FLEDRPLSDKEITIRNEYRKMRDENCKKAKEEFIANNELDVEHLPTHHD (SEQ ID NO: 20)

[00151] In some embodiments, the nucleic acid comprises a *C. jejuni cj0427* gene (provided as SEQ ID NO: 19). In some embodiments, the nucleic acid comprises a *cj0427* gene, wherein the *cj0427* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 19. In some embodiments, the nucleic acid comprises a *cj0427* gene, wherein the *cj0427* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 19.

[00152] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0427 protein, wherein said Cj0427 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 20. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0427 protein, wherein said Cj0427 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 20.

[00153] *cj0428*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0428* gene (*e.g.*, a *C. jejuni cj0428* gene).

[00154] In some embodiments, the nucleic acid comprising a *cj0428* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0428* gene is located on a chromosome of the bacterium.

[00155] The nucleic acid sequence of an exemplary *C. jejuni cj0428* gene is provided below:

ATGCAGGTAAATTATAGAACGATTAGCTCGTATGAATACGATGCTATTAGTGGTCAGTATAA
ACAGGTGGATAAACAGATTGAAGATTATTCTTCATCTGGAGATTCTGATTTTATGGATATGT
TAAATAAGGCGGATGAGAAGTCAAGCGGAGATGCTTTAAATTCTAGCAGTAGTTTTCAAAGC
AATGCGCAAAACTCAAATTCAAATTTAAGTAATTATGCTCAAATGTCAAATGTTTACGCTTA
TCGTTTTAGACAAAATGAAGGCGAGCTGTCTATGAGAGCTCAAAGTGCTAGCGTTCATAATG
ATCTTACACAACAAGGTGCAAATGAACAAAGTAAGAATAATACTTTGTTAAATGATTTATTG
AACGCAATTTAA (SEQ ID NO: 21)

[00156] The amino acid sequence of the Cj0428 protein encoded by the nucleic acid of SEQ ID NO: 21 is provided below:

MQVNYRTISSYEYDAISGQYKQVDKQIEDYSSSGDSDFMDMLNKADEKSSGDALNSSSSFQS
NAQNSNSNLSNYAQMSNVYAYRFRQNEGELSMRAQSASVHNDLTQQGANEQSKNNTLLNDLL
NAI (SEQ ID NO: 22)

[00157] In some embodiments, the nucleic acid comprises a *C. jejuni cj0428* gene (provided as SEQ ID NO: 21). In some embodiments, the nucleic acid comprises a *cj0428* gene, wherein the *cj0428* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 21. In some embodiments, the nucleic acid comprises a *cj0428* gene, wherein the *cj0428* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 21.

[00158] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0428 protein, wherein said Cj0428 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least

84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0428 protein, wherein said Cj0428 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 22.

[00159] *cj0588*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0588* gene (*e.g.*, a *C. jejuni cj0588* gene).

[00160] In some embodiments, the nucleic acid comprising a *cj0588* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0588* gene is located on a chromosome of the bacterium.

[00161] The nucleic acid sequence of an exemplary *C. jejuni cj0588* gene is provided below:

ATGCGTTTCGACTTCTTCGTGTCCAAACGTCTGAACATCAGCCGTAACAAAGCGC
TGGAGCTGATCGAAAACGAAGAGATCCTGCTGAACGGCAAAAGCTTCAAAGCGT
CCTTCGACGTGAAAAACTTCCTGGAAAACCTGAAAAAAACCCAGGACCTGAACC
CGGAAGACATCCTGCTGGCGAACGAGCTGAAACTGGACCTGCTGAGCGAAATCT
ACGTGTCCCGTGCGGCGCTGAAACTGAAAAAATTCCTGGAAGAAAACGACATCG
AAATCAAACACAAAAACTGTCTGGACATCGGCTCCAGCACCGGCGGCTTCGTGC
AGATCCTGCTGGAAAACCAGGCGCTGAAAATCACCGCGCTGGACGTGGGCAGCA
ACCAGCTGCACCCGAGCCTGCGTGTGAACGAAAAAATCATCCTGCACGAAAACA
CCGACCTGCGTGCGTTCAAAAGCGAAGAAAAATTCGAACTGGTGACCTGCGACG
TGAGCTTCATCTCCCTGATCAACCTGCTGTACTACATCGACAACCTGGCGCTGAA
AGAAATCATCCTGCTGTTCAAACCGCAGTTCGAAGTGGGCAAAAACATCAAACG
TGACAAAAAAGGCGTGCTGAAAGACGACAAAGCGATCCTGAAAGCGCGTATGG
ACTTCGAAAAAGCGTGCGCGAAACT (SEQ ID NO: 23)

[00162] The amino acid sequence of the TlyA protein encoded by the nucleic acid of SEQ ID NO: 23 is provided below:

MRFDFFVSKRLNISRNKALELIENEEILLNGKSFKASFDVKNFLENLKKTQDLNPEDIL
L

ANELKLDLLSEIYVSRAALKLKKFLEENDIEIKHKNCLDIGSSTGGFVQILLENQALKI
T
ALDVGSNQLHPSLRVNEKIILHENTDLRAFKSEEKFELVTCDVSFISLINLLYYIDNLA
L
KEIILLFKPQFEVGKNIKRDKKGVLKDDKAILKARMDFEKACAKLGWLLKNTQKSSI
KGKEGNVEYFYYYIKN (SEQ ID NO: 24)

[00163] In some embodiments, the nucleic acid comprises a *C. jejuni cj0588* gene (provided as SEQ ID NO: 23). In some embodiments, the nucleic acid comprises a *cj0588* gene, wherein the *cj0588* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 23. In some embodiments, the nucleic acid comprises a *cj0588* gene, wherein the *cj0588* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 23.

[00164] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a TlyA protein, wherein said TlyA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a TlyA protein, wherein said TlyA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 24.

[00165] *cj0921c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0921c* gene (*e.g.*, a *C. jejuni cj0921c* gene).

[00166] In some embodiments, the nucleic acid comprising a *cj0921c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0921c* gene is located on a chromosome of the bacterium.

[00167] The nucleic acid sequence of an exemplary *C. jejuni cj0921c* gene is provided below:

ATGGTTTTTAGAAAATCTTTGTTAAAGTTGGCAGTTTTTGCTCTAGGTGCTTGTGT
TGCATTTAGCAATGCTAATGCAGCAGAAGGCAAACTGGAGTCCATCAAATCCAA
AGGCCAGCTGATCGTGGGCGTGAAAAACGACGTGCCGCACTACGCTCTGCTGGA
CCAGGCAACCGGCGAAATCAAAGGCTTCGAAGTGGACGTGGCCAAACTGCTGGC
TAAAAGCATCCTGGGGGACGACAAAAAAATCAAACTGGTGGCAGTGAACGCCAA
AACCCGTGGCCCGCTGCTGGACAACGGCAGCGTGGACGCGGTGATCGCAACCTT
CACCATCACCCCGGAGCGCAAACGTATCTATAACTTCTCCGAGCCGTATTATCAG
GACGCTATCGGCCTGCTGGTTCTGAAAGAAAAAAAATATAAATCTCTGGCTGAC
ATGAAAGGTGCAAACATCGGCGTGGCTCAAGCTGCAACTACAAAAAAAGCTATC
GGCGAAGCTGCTAAAAAAATCGGCATCGACGTGAAATTCAGCGAATTCCCGGAC
TATCCGAGCATCAAAGCTGCTCTGGACGCTAAACGTGTGGACGCGTTCTCTGTGG
ACAAATCCATCCTGCTGGGCTATGTGGACGACAAAAGCGAAATCCTGCCGGACA
GCTTCGAACCGCAGAGCTATGGCATCGTGACCAAAAAAGACGACCCGGCTTTCG
CAAAATATGTGGACGACTTCGTGAAAGAACACAAAAACGAAATCGACGCTCTGG
CGAAAAAATGGGGCCTGTAA (SEQ ID NO: 25)

[00168] The amino acid sequence of the Peb1 protein encoded by the nucleic acid of SEQ ID NO: 25 is provided below:

MVFRKSLLKLAVFALGACVAFSNANAAEGKLESIKSKGQLIVGVKNDVPHYALLDQ
ATGEIKGFEVDVAKLLAKSILGDDKKIKLVAVNAKTRGPLLDNGSVDAVIATFTITPE
RKRIYNFSEPYYQDAIGLLVLKEKKYKSLADMKGANIGVAQAATTKKAIGEAAKKIG
IDVKFSEFPDYPSIKAALDAKRVDAFSVDKSILLGYVDDKSEILPDSFEPQSYGIVTKK
DDPAFAKYVDDFVKEHKNEIDALAKKWGL (SEQ ID NO: 26)

[00169] In some embodiments, the nucleic acid comprises a *C. jejuni cj0921c* gene (provided as SEQ ID NO: 25). In some embodiments, the nucleic acid comprises a *cj0921c* gene, wherein the *cj0921c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 25. In some embodiments, the nucleic acid comprises a *cj0921c* gene, wherein the *cj0921c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least

84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 25.

[00170] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Peb1 protein, wherein said Peb1 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 26. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Peb1 protein, wherein said Peb1 protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 26.

[00171] *cj0982c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0982c* gene (*e.g.*, a *C. jejuni cj0982c* gene).

[00172] In some embodiments, the nucleic acid comprising a *cj0982c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0982c* gene is located on a chromosome of the bacterium.

[00173] The nucleic acid sequence of an exemplary *C. jejuni cj0982c* gene is provided below:

ATGAAAAAAATACTTCTAAGTGTTTTAACGGCCTTTGTTGCAGTAGTATTGGCTG
CGGCGGCAACTCCGACTCCAAAACCCTGAACTCCCTGGACAAAATCAAACAGAA
CGGCGTGGTGCGTATCGGCGTGTTTGGCGACAAACCGCCGTTTGGCTATGTGGAC
GAAAAAGGCAACAACCAGGGCTATGACATCGCTCTGGCTAAACGTATCGCGAAA
GAACTGTTTGGCGACGAAAACAAAGTGCAGTTTGTGCTGGTGGAAGCTGCGAAC
CGTGTGGAGTTTCTGAAATCCAACAAAGTGGACATCATCCTGGCTAACTTTACCC
AGACCCCGCAGCGTGCGGAGCAGGTGGACTTTTGCTCCCCGTATATGAAAGTGG
CTCTGGGCGTGGCTGTGCCGAAAGACAGCAACATCACCAGCGTGGAAGACCTGA
AAGACAAAACCCTGCTGCTGAACAAAGGCACCACCGCGGACGCTTATTTTACCC
AGAACTATCCGAACATCAAAACCCTGAAATATGACCAGAACACCGAAACCTTTG
CGGCTCTGATGGACAAACGTGGCGACGCTCTGAGCCACGACAACACCCTGCTGT

TTGCTTGGGTGAAAGACCACCCGGACTTTAAAATGGGCATCAAAGAGCTGGGCA
ACAAAGACGTGATCGCGCCGGCGGTGAAAAAAGGCGACAAAGAACTGAAAGAA
TTTATCGACAACCTGATCATCAAACTGGGCCAGGAGCAGTTTTTTCACAAAGCTT
ATGACGAAACCCTGAAAGCTCACTTTGGCGACGACGTGAAAGCGGACGACGTGG
TGATCGAAGGCGGCAAAATCTAA (SEQ ID NO: 27)

[00174] The amino acid sequence of the CjaA protein encoded by the nucleic acid of SEQ ID NO: 27 is provided below:
MKKILLSVLTAFVAVVLAACGGNSDSKTLNSLDKIKQNGVVRIGVFGDKPPFGYVDE
KGNNQGYDIALAKRIAKELFGDENKVQFVLVEAANRVEFLKSNKVDIILANFTQTPQ
RAEQVDFCSPYMKVALGVAVPKDSNITSVEDLKDKTLLLNKGTTADAYFTQNYPNI
KTLKYDQNTETFAALMDKRGDALSHDNTLLFAWVKDHPDFKMGIKELGNKDVIAP
AVKKGDKELKEFIDNLIIKLGQEQFFHKAYDETLKAHFGDDVKADDVVIEGGKI
(SEQ ID NO: 28)

[00175] In some embodiments, the nucleic acid comprises a *C. jejuni cj0982c* gene (provided as SEQ ID NO: 27). In some embodiments, the nucleic acid comprises a *cj0982c* gene, wherein the *cj0982c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 27. In some embodiments, the nucleic acid comprises a *cj0982c* gene, wherein the *cj0982c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 27.

[00176] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CjaA protein, wherein said CjaA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CjaA protein, wherein said CjaA protein comprises an amino acid sequence that is at least 75%, at least

80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 28.

[00177] *cj0998c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj0998c* gene (*e.g.*, a *C. jejuni cj0998c* gene).

[00178] In some embodiments, the nucleic acid comprising a *cj0998c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj0998c* gene is located on a chromosome of the bacterium.

[00179] The nucleic acid sequence of an exemplary *C. jejuni cj0998c* gene is provided below:

ATGAAAAAAATTCTTGTAAGTGTTTTAAGTTCTTGCTTGTTAGCTTCGGCTTTAAG
TGCGGTGTCCTTCAAAGAAGACAGCCTGAAAATCTCCTTCGAAGGCTACAAAAC
CAAAGACATGATCGGCACCAAAGGCGAATTCAAAAACGTGGAATACAAATTCTC
CAAAAACATCAAAGACCTGGCGAGCTACCTGAAAGGCGCGAAAGCGACCATCA
AACCGAGCAACGCGTTCATGGGCGAAGGCAACGACATCATCACCAACAACATCA
CCAAAGTGTTCTTCCCGGCGCTGCTGGGCGACACGGACATCAAAGTGGTGTTTCA
GGACGTGATCGCGGGCGAAAACAAAGGCGTGATCTCCGCGAAAATCACCATGGA
CAAAAAAAGCACCATCGTGCCGCTGACCTATACCATCAAAGACAACAAATTTGA
AGCGAAAGGCCAGCTGGACCTGCACACCTTTAAAAACGGCTCCAAAGCGCTGAA
AGCGCTGAGCGACGTGGCTGCAGGCCACGGCGGCATCTCCTGGCCGCTGGTGGA
CATCAGCTTTAACGCGGACCTGGCGGAATAA (SEQ ID NO: 29)

[00180] The amino acid sequence of the Cj0998c protein encoded by the nucleic acid of SEQ ID NO: 29 is provided below:

MKKILVSVLSSCLLASALSAVSFKEDSLKISFEGYKTKDMIGTKGEFKNVEYKFSKNI
KD
LASYLKGAKATIKPSNAFMGEGNDIITNNITKVFFPALLGDTDIKVVFQDVIAGENKG
VI
SAKITMDKKSTIVPLTYTIKDNKFEAKGQLDLHTFKNGSKALKALSDVAAGHGGISW
PLVDISFNADLAE (SEQ ID NO: 30)

[00181] In some embodiments, the nucleic acid comprises a *C. jejuni cj0998c* gene (provided as SEQ ID NO: 29). In some embodiments, the nucleic acid comprises a *cj0998c* gene, wherein the *cj0998c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least

86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 29. In some embodiments, the nucleic acid comprises a *cj0998c* gene, wherein the *cj0998c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 29.

[00182] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0998c protein, wherein said Cj0998c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj0998c protein, wherein said Cj0998c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 30.

[00183] *cj1259*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj1259* gene (*e.g.*, a *C. jejuni cj1259* gene).

[00184] In some embodiments, the nucleic acid comprising a *cj1259* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj1259* gene is located on a chromosome of the bacterium.

[00185] The nucleic acid sequence of an exemplary *C. jejuni cj1259* gene is provided below:

ATGAAACTAGTTAAACTTAGTTTAGTTGCAGCTCTTGCTGCAGGTGCTTTTTCAGC
AGCTAACGCTACCCCGCTGGAAGAAGCGATCAAAGACGTGGACGTGTCCGGCGT
GCTGCGTTACCGTTACGACACCGGCAACTTTGACAAAAACTTCGTGAACAACTCC
AACCTGAACAACAGCAAACAGGACCACAAATATCGTGCACAGGTGAACTTCAGT
GCTGCTATCGCTGACAACTTCAAAGCTTTTGTGCAGTTTGACTATAACGCTGCTG
ACGGTGGCTATGGCGCTAACGGCATCAAAAACGACCAGAAAGGCCTGTTTGTGC

GTCAGCTGTACCTGACTTATACCAACGAAGACGTGGCTACCAGTGTGATCGCTGG
TAAACAGCAGCTGAACCTGATCTGGACGGACAACGCTATCGACGGTCTGGTGGG
CACCGGTGTGAAAGTGGTGAACAACAGCATCGACGGTCTGACTCTGGCTGCTTTT
GCTGTGGACAGCTTCATGGCTGCGGAGCAGGGTGCGGACCTGCTGGAACACAGT
AACATCTCCACCACCTCCAACCAGGCTCCGTTTAAAGTGGACTCCGTGGGCAACC
TGTACGGTGCTGCTGCTGTGGGTTCTTATGACCTGGCTGGTGGCCAGTTCAACCC
GCAGCTGTGGCTGGCTTATTGGGACCAGGTGGCATTCTTCTATGCTGTGGACGCA
GCTTATAGCACAACTATCTTTGACGGCATCAACTGGACACTGGAAGGCGCTTACC
TGGGAAACAGCCTGGACAGCGAACTGGACGACAAAACACACGCTAACGGCAAC
CTGTTTGCTCTGAAAGGCAGCATCGAAGTGAACGGCTGGGACGCTAGCCTGGGT
GGTCTGTACTACGGCGACAAAGAAAAAGCTTCTACAGTGGTGATCGAAGACCAG
GGTAACCTGGGTTCTCTGCTGGCAGGTGAGGAAATCTTCTATACTACTGGCTCAC
GCCTGAACGGTGACACTGGTCGTAACATCTTCGGTTATGTGACTGGTGGATATAC
TTTCAACGAAACAGTGCGCGTGGGTGCTGACTTCGTGTATGGTGGAACAAAAAC
AGAAGCTGCTAACCACCTGGGTGGTGGTAAAAAACTGGAAGCTGTGGCACGCGT
GGACTACAAATACTCTCCGAAACTGAACTTCTCAGCATTCTATTCTTATGTGAAC
CTGGACCAGGGTGTGAACACTAACGAAAGTGCTGACCACAGCACTGTGCGTCTG
CAGGCTCTGTACAAATTCTAA (SEQ ID NO: 31)

[00186] The amino acid sequence of the PorA protein encoded by the nucleic acid of SEQ ID NO: 31 is provided below:

MKLVKLSLVAALAAGAFSAANATPLEEAIKDVDVSGVLRYRYDTGNFDKNFVNNS
NLNNSKQDHKYRAQVNFSAAIADNFKAFVQFDYNAADGGYGANGIKNDQKGLFVR
QLYLTYTNEDVATSVIAGKQQLNLIWTDNAIDGLVGTGVKVVNNSIDGLTLAAFAV
DSFMAAEQGADLLEHSNISTTSNQAPFKVDSVGNLYGAAAVGSYDLAGGQFNPQL
WLAYWDQVAFFYAVDAAYSTTIFDGINWTLEGAYLGNSLDSELDDKTHANGNLFA
LKGSIEVNGWDASLGGLYYGDKEKASTVVIEDQGNLGSLLAGEEIFYTTGSRLNGDT
GRNIFGYVTGGYTFNETVRVGADFVYGGTKTEAANHLGGGKKLEAVARVDYKYSP
KLNFSAFYSYVNLDQGVNTNESADHSTVRLQALYKF (SEQ ID NO: 32)

[00187] In some embodiments, the nucleic acid comprises a *C. jejuni cj1259* gene (provided as SEQ ID NO: 31). In some embodiments, the nucleic acid comprises a *cj1259* gene, wherein the *cj1259* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%,

at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 31. In some embodiments, the nucleic acid comprises a *cj1259* gene, wherein the *cj1259* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 31.

[00188] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PorA protein, wherein said PorA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 32. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PorA protein, wherein said PorA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 32.

[00189] *cj1339c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj1339c* gene (*e.g.*, a *C. jejuni cj1339c* gene).

[00190] In some embodiments, the nucleic acid comprising a *cj1339c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj1339c* gene is located on a chromosome of the bacterium.

[00191] The nucleic acid sequence of an exemplary *C. jejuni cj1339c* gene is provided below:

ATGGGTTTTCGTATCAACACCAACGTGGCGGCTCTGAACGCAAAAGCAAACGCG
GATCTGAACAGCAAAAGCCTGGATGCTTCTCTGAGCCGTCTGAGCTCCGGCCTGC
GTATCAACTCCGCAGCAGATGATGCTTCCGGGATGGCGATCGCAGATAGCCTGC
GTTCTCAGGCTAACACTCTGGGCCAGGCTATCTCTAACGGCAACGATGCTCTGGG
CATCCTGCAGACTGCTGATAAAGCTATGGACGAGCAGCTGAAAATCCTGGATAC
CATCAAAACTAAAGCAACCCAGGCGGCTCAGGATGGCCAGAGCCTGAAAACCCG
TACCATGCTGCAGGCAGATATCAACCGTCTGATGGAAGAACTGGACAACATCGC

AAACACTACTTCCTTTAACGGTAAACAGCTGCTGAGCGGCAACTTTATCAACCAG
GAATTTCAGATCGGCGCAAGCTCCAACCAGACTGTGAAAGCTACTATCGGCGCA
ACTCAGTCTTCTAAAATCGGTCTGACCCGCTTTGAAACCGGCGGCCGTATCTCCA
CTAGCGGCGAAGTGCAGTTTACTCTGAAAAACTACAACGGTATCGATGATTTTCA
GTTTCAGAAAGTGGTGATCTCCACTTCCGTGGGCACCGGCCTGGGCGCTCTGGCA
GATGAGATCAACAAAAACGCTGATAAAACCGGTGTGCGTGCTACTTTTACAGTG
GAAACTCGTGGTATCGCTGCAGTGCGTGCAGGCGCTACTTCAGATACTTTTGCTA
TCAACGGGGTGAAAATCGGCAAAGTGGATTACAAAGATGGCGATGCTAACGGCG
CCCTGGTGGCTGCAATCAACTCGGTGAAAGATACCACCGGCGTGGAAGCTTCGA
TCGATGCTAACGGCCAGCTGCTGCTGACTTCCCGTGAAGGCCGTGGCATCAAAAT
CGATGGTAACATCGGTGGCGGTGCCTTTATCAACGCTGATATGAAAGAAAACTAT
GGCCGCCTGTCTCTGGTGAAAAACGATGGTAAAGATATCCTGATCAGCGGTAGC
AACCTGTCTTCTGCAGGTTTTGGTGCAACCCAGTTTATCTCTCAGGCTTCTGTGTC
TCTGCGTGAGTCCAAAGGCCAGATCGATGCTAACATCGCTGATGCTATGGGCTTT
GGCTCTGCAAACAAAGGCGTGGTGCTGGGTGGTTATTCTTCTGTGAGCGCCTATA
TGAGCAGCGCAGGCAGCGGCTTTTCTTCCGGTTCCGGTTATTCTGTGGGTAGCGG
CAAAAACTATTCCACCGGTTTTGCAAACGCTATCGCTATCTCCGCTGCTTCGCAG
CTGTCTACGGTGTATAACGTGTCTGCAGGCTCAGGTTTTTCAAGCGGTTCCACCCT
GTCTCAGTTTGCCACTATGAAAACCACTGCTTTTGGCGTGAAAGATGAAACCGCA
GGTGTGACCACCCTGAAAGGCGCTATGGCTGTGATGGATATCGCTGAAACCGCT
ATCACCAACCTGGATCAGATCCGTGCCGACATCGGCTCGGTGCAGAACCAGGTG
ACATCCACTATCAACAACATCACCGTGACTCAGGTGAACGTGAAAGCAGCAGAA
TCGCAGATCCGTGATGTGGACTTTGCAGCCGAGAGCGCAAACTACTCTAAAGCA
AACATCCTGGCTCAGAGCGGCTCTTATGCCATGGCACAGGCTAACTCTGTGCAGC
AGAACGTGCTGCGTCTGCTGCAGTA (SEQ ID NO: 33)

[00192] The amino acid sequence of the FlaA protein encoded by the nucleic acid of SEQ ID NO: 33 is provided below:

MGFRINTNVAALNAKANADLNSKSLDASLSRLSSGLRINSAADDASGMAIADSLRSQ
ANTLGQAISNGNDALGILQTADKAMDEQLKILDTIKTKATQAAQDGQSLKTRTMLQ
ADINRLMEELDNIANTTSFNGKQLLSGNFINQEFQIGASSNQTVKATIGATQSSKIGLT
RFETGGRISTSGEVQFTLKNYNGIDDFQFQKVVISTSVGTGLGALADEINKNADKTGV
RATFTVETRGIAAVRAGATSDTFAINGVKIGKVDYKDGDANGALVAAINSVKDTTG
VEASIDANGQLLLTSREGRGIKIDGNIGGGAFINADMKENYGRLSLVKNDGKDILISG

SNLSSAGFGATQFISQASVSLRESKGQIDANIADAMGFGSANKGVVLGGYSSVSAYM
SSAGSGFSSGSGYSVGSGKNYSTGFANAIAISAASQLSTVYNVSAGSGFSSGSTLSQF
ATMKTTAFGVKDETAGVTTLKGAMAVMDIAETAITNLDQIRADIGSVQNQVTSTINN
ITVTQVNVKAAESQIRDVDFAAESANYSKANILAQSGSYAMAQASVQQNVLRLLQ
(SEQ ID NO: 34)

[00193] In some embodiments, the nucleic acid comprises a *C. jejuni cj1339c* gene (provided as SEQ ID NO: 33). In some embodiments, the nucleic acid comprises a *cj1339c* gene, wherein the *cj1339c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 33. In some embodiments, the nucleic acid comprises a *cj1339c* gene, wherein the *cj1339c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 33.

[00194] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a FlaA protein, wherein said FlaA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 34. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a FlaA protein, wherein said FlaA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 34.

[00195] *cj1478c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj1478c* gene (*e.g.*, a *C. jejuni cj1478c* gene).

[00196] In some embodiments, the nucleic acid comprising a *cj1478c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj1478c* gene is located on a chromosome of the bacterium.

[00197] The nucleic acid sequence of an exemplary *C. jejuni cj1478c* gene is provided below:

ATGAAAAAAATCTTCCTGTGTCTGGGCCTGGCGAGCGTGCTGTTTGGCGCTGACA
ACAACGTGAAATTTGAAATCACCCCGACCCTGAACTATAACTACTTTGAAGGCAA
CCTGGACATGGACAACCGTTATGCGCCGGGGATCCGTCTGGGCTATCACTTTGAC
GACTTTTGGCTGGACCAGCTGGAATTTGGGCTGGAGCACTATTCTGACGTGAAAT
ATACCAACACCAACAAAACCACCGACATCACCCGTACCTATCTGAGCGCTATCA
AAGGCATCGACGTGGGTGAGAAATTTTATTTCTATGGCCTGGCAGGCGGCGGCTA
TGAGGACTTTTCCAACGCTGCGTATGACAACAAAAGCGGCGGCTTTGGCCACTAT
GGCGCGGGCGTGAAATTCCGTCTGAGCGACTCTCTGGCTCTGCGTCTGGAAACCC
GTGACCAGATCAACTTCAACCACGCAAACCACAACTGGGTGTCCACTCTGGGCA
TCAGCTTTGGCTTTGGCGGCAAAAAAGAAAAAGCTGTGGAAGAAGTGGCTGACA
CCCGTGCAACTCCGCAGGCCAAATGTCCGGTGGAACCGCGTGAAGGCGCTCTGC
TGGACGAAAACGGCTGCGAAAAAACCATCTCTCTGGAAGGCCACTTTGGCTTTG
ACAAAACCACCATCAACCCGACTTTTCAGGAAAAAATCAAAGAAATCGCAAAAG
TGCTGGACGAAAACGAACGTTATGACACTATCCTGGAAGGCCACACCGACAACA
TCGGCTCCCGTGCTTATAACCAGAAACTGTCCGAACGTCGTGCTAAAAGCGTGGC
TAACGAACTGGAAAAATATGGCGTGGAAAAAAGCCGCATCAAAACAGTGGGCTA
TGGCCAGGACAACCCGCGCTCCAGCAACGACACCAAAGAAGGCCGCGCGGACA
ACCGTCGCGTGGACGCTAAATTTATCCTGCGCTAA (SEQ ID NO: 35)

[00198] The amino acid sequence of the CadF protein encoded by the nucleic acid of SEQ ID NO: 35 is provided below:

MKKIFLCLGLASVLFGADNNVKFEITPTLNYNYFEGNLDMDNRYAPGIRLGYHFDDF
WLDQLEFGLEHYSDVKYTNTNKTTDITRTYLSAIKGIDVGEKFYFYGLAGGGYEDFS
NAAYDNKSGGFGHYGAGVKFRLSDSLALRLETRDQINFNHANHNWVSTLGISFGFG
GKKEKAVEEVADTRATPQAKCPVEPREGALLDENGCEKTISLEGHFGFDKTTINPTF
QEKIKEIAKVLDENERYDTILEGHTDNIGSRAYNQKLSERRAKSVANELEKYGVEKS
RIKTVGYGQDNPRSSNDTKEGRADNRRVDAKFILR (SEQ ID NO: 36)

[00199] In some embodiments, the nucleic acid comprises a *C. jejuni cj1478c* gene (provided as SEQ ID NO: 35). In some embodiments, the nucleic acid comprises a

cj1478c gene, wherein the *cj1478c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 35. In some embodiments, the nucleic acid comprises a *cj1478c* gene, wherein the *cj1478c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 35.

[00200] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CadF protein, wherein said CadF protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 36. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a CadF protein, wherein said CadF protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 36.

[00201] *cj1534c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj1534c* gene (*e.g.*, a *C. jejuni cj1534c* gene).

[00202] In some embodiments, the nucleic acid comprising a *cj1534c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj1534c* gene is located on a chromosome of the bacterium.

[00203] The nucleic acid sequence of an exemplary *C. jejuni cj1534c* gene is provided below:

ATGTCCGTGACCAAACAGCTGCTGCAGATGCAGGCGGACGCGCACCACCTGTGG
GTGAAATTCCACAACTACCACTGGAACGTGAAAGGCCTGCAGTTCTTCTCCATCC
ACGAGTACACCGAAAAAGCGTACGAAGAAATGGCAGAACTGTTCGACAGCTGTG
CGGAACGTGTGCTGCAGCTGGGCGAAAAAGCGATCACCTGCCAGAAAGTGCTGA

TGGAAAACGCGAAAAGCCCGAAAGTGGCGAAAGACTGCTTCACCCCGCTGGAAG
TGATCGAACTGATCAAACAGGACTACGAATACCTGCTGGCGGAATTCAAAAAAC
TGAACGAAGCGGCAGAAAAAGAAAGCGACACCACCACCGCTGCTTTCGCGCAGG
AAAACATCGCGAAATATGAAAAAAGTCTGTGGATGATCGGCGCTACCCTGCAGG
GCGCTTGCAAAATGTAA (SEQ ID NO: 37)

[00204] The amino acid sequence of the Dps protein encoded by the nucleic acid of SEQ ID NO: 37 is provided below:
MSVTKQLLQMQADAHHLWVKFHNYHWNVKGLQFFSIHEYTEKAYEEMAELFDSC
AERVLQLGEKAITCQKVLMENAKSPKVAKDCFTPLEVIELIKQDYEYLLAEFKKLNE
AAEKESDTTTAAFAQENIAKYEKSLWMIGATLQGACKM (SEQ ID NO: 38)

[00205] In some embodiments, the nucleic acid comprises a *C. jejuni cj1534c* gene (provided as SEQ ID NO: 37). In some embodiments, the nucleic acid comprises a *cj1534c* gene, wherein the *cj1534c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 37. In some embodiments, the nucleic acid comprises a *cj1534c* gene, wherein the *cj1534c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 37.

[00206] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Dps protein, wherein said Dps protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 38. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Dps protein, wherein said Dps protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,

at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 38.

[00207] *cj1656c*– In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *cj1656c* gene (*e.g.*, a *C. jejuni cj1656c* gene).

[00208] In some embodiments, the nucleic acid comprising a *cj1534c* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *cj1534c* gene is located on a chromosome of the bacterium.

[00209] The nucleic acid sequence of an exemplary *C. jejuni cj1656c* gene is provided below:

ATGGTTTCAGATGTTTCTATGGGTAATGTTAATTTAATGACTGCTGTTAATACTTCAGTTTT
GAAAAAATCTATGGACACAAACGAGGCATTGATGAATGAACTCATCGAAGGTATGGAAGGTG
TCTCTCAAGCCTCCGCTCCACAAGCTTCTAGCTCTAGTGGTTTGGATATTTACGCTTAA
(SEQ ID NO: 39)

[00210] The amino acid sequence of the Cj1656c protein encoded by the nucleic acid of SEQ ID NO: 39 is provided below:

MVSDVSMGNVNLMTAVNTSVLKKSMDTNEALMNELIEGMEGVSQASAPQASSSSGLDIYA
(SEQ ID NO: 40)

[00211] In some embodiments, the nucleic acid comprises a *C. jejuni cj1656c* gene (provided as SEQ ID NO: 39). In some embodiments, the nucleic acid comprises a *cj1656c* gene, wherein the *cj1656c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 39. In some embodiments, the nucleic acid comprises a *cj1656c* gene, wherein the *cj1656c* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO:39.

[00212] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj1656c protein, wherein said Cj1656cs protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 40. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Cj1656c protein, wherein said Cj1656c protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 40.

B. *Campylobacter* Glycosylation Proteins

[00213] In some embodiments, the recombinant bacterium has been genetically engineered to produce the *Campylobacter jejuni* N-glycan in its surface. The *C. jejuni* N-glycan is a heptasaccharide ((GalNAc-α1,4-GalNAc-α1,4-[Glc-β-1,3]GalNAc-α1,4-GalNAc-α1,4-GalNAc-α1,3-diNAcBac; diNAcBac is 2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose, GalNAc is N-acetylgalactosamine and Glc is glucose), and is commonly found in all *C. jejuni* and *Campylobacter coli* isolates. In *Campylobacter*, the N-glycan is added to multiple periplasmic and membrane proteins, and is immunogenic in rabbits and humans (*see*, *e.g.*, Nothaft *et al.* (2012) *Mol. Cell. Proteomics* 11: 1203-19 (13); and Szymanski *et al.* (2003) *J Biol Chem.* 278: 24509-20 (14)).

[00214] The *C. jejuni* N-glycan is conjugated onto the asparagine within the Glu/Asp Xaa1 Asn-Xaa2-Ser/Thr (SEQ ID NO: 41) glycosylation consensus motif. In *C. jejuni*, the genes encoding proteins that mediate N-linked glycosylation are present in a 17-kb locus named the *pgl* operon which contains 14 open reading frames (ORFs) (*see* Wacker *et al.* (2002) *Science* 298: 1790-3(15)). The 14 ORFs encode several glycotransferases and sugar biosynthetic enzymes which in concert generate a lipid-linked heptasaccharide precursor. The heptasaccharide is transferred onto the target asparagine by PglB in the bacterial periplasm. The *C. jejuni pgl* operon comprises the following genes: *wlaA*, *gne*, *pglK*, *pglH*, *pglI*, *pglJ*, *pglB*, *pglA*, *pglC*, *pglD*, *wlaJ*, *pglE*, *pglF*, and *pglG* (**Fig. 6**).

Table A: *pgl* operon gene size and GC contents

gene	aa	bp	GC %
wlaA	264	795	29.6
gne	330	993	34.0

pglK	526	1581	29.0
pglH	354	1062	30.7
pglI	304	912	29.3
pglJ	362	1089	29.6
pglB	713	2142	29.2
pglA	376	1128	30.8
pglC	198	597	31.7
pglD	195	588	31.1
wlaJ	217	654	24.3
pglE	386	1161	31.0
pglF	590	1770	30.7
pglG	297	894	30.1

[00215] In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pgl* operon (*e.g.*, a *C. jejuni pgl* operon or a *C. coli pgl* operon). In some embodiments, the recombinant bacterium comprises at least one *pgl* operon gene selected from the group consisting of *walA, gne, pglK, pglH, pglI, pglJ, pglB, pglA, pglC, pglD, wlaJ, pglE, pglF,* and *pglG.* Expression of the nucleic acid comprising the *pgl* operon or of one or more of the *pgl* operon genes may confer the bacterium (e.g., a non-*Campylobacter* bacterium) the ability to synthesize and N-glycan and conjugate the N-glycan to proteins comprising the Glu/Asp Xaa1 Asn-Xaa2-Ser/Thr (SEQ ID NO: 41) glycosylation consensus motif.

[00216] In some embodiments, the nucleic acid comprising a *pgl* operon is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pgl* operon is located on a chromosome of the bacterium. In some embodiments, the nucleic acid comprising a *pgl* operon is located at the chromosomal locus corresponding to the locus of an endogenous *cysG* gene that has been deleted or altered in the bacterial chromosome. In some embodiments, the nucleic acid comprising a *pgl* operon is operably linked to a regulatable promoter (*e.g.*, a LacI-regulatable promoter such as P_{trc}).

[00217] The nucleic acid sequence of an exemplary *C. jejuni pgl* operon is provided below:

GGATTACAAATGGCAAAAAATGAAGGTTATATTTGTGTTTTTGATTGTGAGAGTGTGCCAGATGTTGAGCTTATC
CGCAAAACTTTGGGTTTTGAAGGAAGTGATTTAGAGGTAAGTTTAAAAGCACTTCAGTGGCAAAAAGAACAAAGT
GGGAGTGAGTTTTTGCCTTTGCCTTATCATAAAATTATCAGTATTTGTGCGGTTTTAAGTGATAATTTTGGAAAA
TTTATCAAAGTGAATAAAATTGATGGACAAAATGAAAAAGAAATGATTGAGAATTTTTTCAATTTTATAGAAAAT
TATGAGCCAAAATTAGTCAGTTTTAATGGTAAAAATTTCGATATGCCTGTTCTTGTTTTAAGGGCTTTAAAATAC
AATTTAAAAGCAGCAACTTATTTGGATACTCAAAGTGATAAATGGAATAATTATAAAACAAGATTTTCAGAATTA
AAACATTGTGATTTATTAGAATCCTTAGGATCTAACGGGCGTGGAATAAAGCTTGATACACTTTGTTCTATGGTG
GGTTTGCCAGGAAAATATGATGTGCATGGCGATGAGGTAATGAAACTTTTTTATGAAAATAAACTTGAAAAAATC
CACGAATATTGTGAAAGTGATGTTTTAAACACCTATATGCTTTTTTTAAAATATGAACTTATTAAAGCTAATGTT
GATGAAGAAGATTATGTTGGTTTTCTTTCTTATATGAGAGATTTCTTGTGTGCAAAAAAATCAGATCGTTCTTAT

ACAGAAGTTTTTGCAAAAGCTTGTGAGAGTGAAATTTCAAAAGTTCGATCTTAAGTATTTAAGAAAATATATTAA
AATTTATTTTTGACATTTTTAAAAAAAGGAATGATGATGAAAATTCTTATTAGCGGTGGTGCAGGTTATATAGGT
TCTCATACTTTAAGACAATTTTTAAAAACAGATCATGAAATTTGTGTTTTAGATAATCTTTCTAAGGGTTCTAAA
ATCGCAATAGAAGATTTGCAAAAAACAAGAGCTTTTAAATTTTTCGAACAAGATTTAAGTGATTTTCAAGGCGTA
AAAGCATTGTTTGAGAGAGAAAAATTTGACGCTATTGTGCATTTTGCAGCAAGCATTGAAGTTTTTGAAAGTATG
CAAAATCCTTTAAAATATTATATGAACAACACTGTTAATACGACAAATCTCATCGAAACTTGTTTGCAAACTGGA
GTGAATAAATTTATATTTTCTTCAACGGCGGCCACTTATGGCGAACCACAAACTCCCGTTGTGAGCGAAACAAGT
CCTTTAGCACCTATTAATCCTTATGGGCGTAGTAAGCTTATGAGTGAAGAAGTTTTGCGTGATGCAAGTATGGCA
AATCCTGAATTTAAGCATTGTATTTTAAGATATTTTAATGTTGCAGGTGCTTGTATGGATTATACTTTAGGACAA
CGCTATCCAAAAGCGACTTTGCTTATAAAAGTTGCAGCTGAATGTGCCGCAGGAAAACGTGATAAACTTTTCATA
TTTGGCGATGATTATGATACAAAAGATGGTACTTGCATAAGAGATTTTATCCATGTAGATGATATTTCAAGTGCA
CATTTAGCGGCTTTGGATTATTTAAAAGAGAATGAAAGCAATGTTTTTAATGTAGGTTATGGACATGGTTTTAGC
GTAAAAGAAGTGATTGAAGCGATGAAAAAAGTTAGCGGAGTGGATTTTAAAGTAGAACTTGCCCCACGCCGTGCG
GGTGATCCTAGTGTATTGATTTCTGATGCAAGTAAAATCAGAAATCTTACTTCTTGGCAGCCTAAATATGATGAT
TTAGAGCTTATTTGTAAATCTGCTTTTGATTGGGAAAAACAGTGTTAAAAAAACTTTTTTTTATTTTAAGTAAGG
AAGATAAAAATTTTTTATTTTTCTTGCTTGTTTTTTCAGTATTTATTTCTTTTATAGAAACTTTTGCAATTTCTT
TGGTAATGCCTTTTATCACTTTGGCTAGTGATTTTTCTTATTTTGATCGTAATAAATATTTAATCAGCCTAAAAG
AATATCTTAATATCCCTGTTTTTGAAATCATTGTTTATTTTGGAGTGGGGCTTATTGTTTTTTATGTGTTTAGAG
CTTTGTTAAATGCGTATTATTTTCATCTTTTGGCAAGATTTTCTAAAGGGCGTTATCATGCGATCGCTTATAAGG
TTTTTTCTAAATTTTTAAATATTAATTATGAAAAATTTACTCAAAAAAATCAATCTGAAATTTTAAAGTCCATTA
CAGGGGAAGTTTATAATCTAAGCACTATGATTTCATCATTTTTACTTTTGATGAGTGAAATTTTTGTAGTACTTT
TGCTTTATGCTTTAATGCTTTTGATTAATTATAAAATCACTTTGTTTTTAAGTATTTTTATGGTGTTAAATGCCT
TTATTTTAGTGAAAATTTTAAGCCCTATCATTAAAAAAGCAGGAGTAAGACGCGAAGAAGCGATGAAAAATTTCT
TTGAAATTTTAAATACAAATTTAAATAATTTCAAATTTATTAAGCTTAAAACCAAAGAAGATGGAGTATTAAGTC
TTTTTAAAGCGCAAAGTGAAGCTTTTTCTAAAGCAAATATTACCAACGAAAGCGTAGCTGCGGTGCCTAGAATTT
ATCTTGAAGGAATAGGCTTTTGCGTACTTGTTTTTTATCGTGGTATTTTTGGTTTTGAAAAATGAAAGTGATATTT
CAGGTATTTTATCCACGATTTCTATTTTTGTTTTAGCGCTTTATCGCTTAATGCCAAGTGCAAATCGTATTATTA
CAAGTTATCATGATTTGCTTTATTATCATTCTTCTTTGGATATTATTTATCAAAATTTAAGACAAGAAGAAGAAA
ATTTGGGCGAGGAAAAATTAAGCTTTAATCAAGAGCTTAAAATTTGCAATCTTAGCTTTGGTTATGAGGGAAAAA
AATATTTATTTAAAAATCTTAACTTAAATATTAAAAAAGGCGAAAAAATCGCTTTTATAGGGGAGAGTGGTTGTG
GAAAAAGTACCTTAGTAGATCTTATCATAGGACTTTTAAAACCAAAAGAAGGGCAAATTTTAATTGATGAGCAAG
AATTAAATGCAAATAATACAAAAAATTATCGCCAAAAAATAGGCTATATCCCGCAAAATATCTATCTTTTTAATG
ACAGTATAGCTAAAAATATCACTTTTGGAGATGCGGTTGATGAAGAAAAACTTAATAGGGTTATCAAACAAGCAA
ATTTAGAGCATTTTATAAAAAATTTACCTCAAGGAGTGCAAACAAAAGTGGGCGATGGGGGGAGTAATTTAAGCG
GGGGACAAAAACAACGCATAGCTATAGCAAGAGCTTTATATTTAGAGCCTGAAATGTTAGTGCTTGATGAAGCAA
CTTCTGCGCTTGATACTCAAAGTGAAGCAAAAATTATGGATGAAATTTATAAAATTTCTAAAGATAAAACCATGA
TTATTATCGCACATCGCCTTTCTACGATAACACAATGTGATAAGGTTTATCGTTTAGAACACGGTAAGCTTAAAG
AGGAGAAATGATGAAAATAAGCTTTATTATCGCAACTTTAAATTCAGGAGGTGCTGAGCGTGCTTTAGTAACCTT
AGCTAATGCACTTTGCAAAGAGCATGAAGTAAGTATTATTAAATTTCATGCAGGAGAATCTTTTTATAAGCTTGA
AAATGAAGTTAAAGTTACAAGTTTGGAACAATTTAGATTTGACACGCTTTATCATAAAATCGCAAGTCGTTTTAA
GAAATTTTTTGCTTTAAGAAAGGCTTTGAAAGAAAGTAAGTCTGATGTTTTTATTTCTTTTTTGGATACGACTAA
TATTGCTTGTATTGCTGCGAAAATAGGGCTTAAAACTCCACTCATTATAAGTGAGCATAGCAATGAAGCGTATTT
AAAACCTAAAATTTGGCGTTTTTTAAGAAGGGTAAGCTATCCTTTTTGTGATGCTTTAAGTGTGCTTGGAAGCAG
TGATAAGGTGTATTATGAAAGATTTGTAAAAAGGGTTAAGCTTTTATTAAACCCTTGTCATTTTAGCGATGAAAT
TTCTTTTGATTCTAGTTTTGAAAAGGAAAATTTGGTTCTTTTTATAGGGCGTTTAGATCACAACAAAAACCCTGT
AATGTTTTTAAAAGCTATAGCGCATTTGGATAAAAATTTACAAGAAAATTATAAATTTGTTATAGCAGGAGATGG
ACAGTTAAGACAAGAACTTGAATATAAGGTAAAATCTTTAGGAATAAAAGTTGATTTTTTAGGACGCGTTGAAAA
TGTCAAGGCTCTTTATGAAAAAGCAAAAGTGCTTTGCCTTTGTTCTTTTGTAGAGGGTTTGCCAACGGTTTTAAT
TGAAAGTTTGTATTTTGAGGTTTGTAGAATTTCAAGTTCTTATTATAATGGTGCTAAGGATTTAATCAAAGATAA
TCATGATGGGCTTTTGGTAGGTTGTGATGATGAAATAGCACTTGCTAAAAAACTTGAACTTGTTTTAAATGATGA
AAATTTTAGAAAAGAACTTGTAAATAATGCCAAACAAAGGTGTAAAGACTTTGAAATTTCTCATATCAAAGAAGA
ATGGCTTAAGCTTATAGCCGAGGTTAAAAATGCCTAAACTTTCTGTTATAGTACCAACTTTTAATCGTCAAGTTT
TGTTAGAAAAGGCTATTAAAAGCATACAAAATCAAGATTTTAAAGATTTAGAAATTATTGTAAGCGATGATAATT
CTAGCGATGATACTAAAAGTGTGGTGCAAAATTTACAAAAAGATGATGATCGCATTAAGTATTTTTTAAATCAAA
ATTACAAACAAGGTCCAAATGGCAATAAAAACAATGGCTTAGATCAAGCAAGTGGCGAGTTTGTAACTTTTTTAG
ATGATGATGATGAGCTTTTATCCGGGGCTTTAAGTACCTTGATGCAAAAAGCAAATGAGGGTTATGCTCATGTTT
TTGGAAATTGTTTGATAGAAAAAGAAGGAAATTTAAGCAAGGAATTTAGCGGCAAGGGCTTGGAAAAAGATAGTG
AAATTTCTAAAAAAGATTTTTTAATGGCTAAATTTAGCGGAGAGTTTTTTTCTGTTTTTAAAAAATCCCTACTTG
AAAATAAGCGTTTTAATGAAGAATTTTATGGCAATGAAGCCACGCTTTGGGTAAATTTATACAAAGAAAAAAGTT
TTTATATCCATAAGGCTTTTAGGATTTATAGAATTTTTAGGCAAGATAGCGTGACTTTAGGGGCGAGTAAAAATG

CTTATAGGGTGTATTTGGGATATTTAGAGCTTGCTAAAATTTTAGAAAATGAACTTAGAATGAGTAAGGATAAAG
ATTATAAAAAAACTTGTGCGAGTTATTATAAAATGGCAGCTTATTATGCAAAACTTGCAAAAAATTATAAAGCCC
TTTATAAATGTTTGTTTAAAAGCCTAAGTATAAAAATCAACGCTCCTGCTTTGATATTACTCATTTTAAGTATAA
TTCCAAATAATATGATTGAAAAATTATCAAAAATTCGGGTGGCTTTATGCAAAAATTAGGCATTTTTATTTATTC
TTTAGGAAGTGGTGGTGCTGAAAGAGTTGTGGCGACTTTATTGCCTATTTTAAGTTTGAAATTTGAAGTGCATTT
GATCTTGATGAATGATAAAATTTCTTATGAAATTCCAGAGTGTCAAATTCATTTTTTAGAATGTTCAAAACCTAG
TGAAAATCCTATTTTGAAATTTTTAAAACTACCTTTTTTGGCTTTAAAATACAAAAAACTTTGCAGAAATTTAGG
TATTGATACAGAATTTGTTTTTTTAAATCGACCTAATTATATAGCTTTAATGGCAAGAATGTTTGGAAACAAAAC
TCGCCTTGTGATCAATGAATGCACTACGCCAAGTGTGATGTATATGAAAAATAATTTTAATTCTTTGGTAAATAA
ATTTTTAATTTCTTTGCTTTACCCAAAAGCTGATTTAATCTTGCCTAATTCTAAGGGAAATTTAGAAGATTTAGT
GCAAAATTTTAGTATAAGTCCAAAAAAATGTGAAATTTTATACAATGCCATCGATTTAGAAAACATAGGGCAAAA
AGCCCTTGAAGACATAGCTTTAAAAGATAAATTTATTTTAAGTGTAGGCAGGCTTGATAAAGGTAAAAATCATGC
TTTATTAATTCGTGCTTATGCGAGATTGAAAACAGATTTAAAGCTTGTGATTTTAGGTGAAGGTGTGCTTAAGGA
TGAGCTTTTAGCTTTGATTAAAGAATTAAATTTGGAAGAAAAGGTTTTGCTTTTAGGATTTGATAATAATCCTTA
TAAATACATGGCTAAATGCGAATTTTTTGCTTTTGCTTCTGTGTTTGAAGGTTTTTCAAATGTTTTAATCGAAAG
TTTGGCTTGTTCTTGTGCGGTGGTTTGCACTGATCATAAAAGTGGTGCAAGAGAGCTTTTTGGCGATGATGAATT
TGGACTTTTAGTAGAAGTAGATAATGAAAACTCTATGTTTCAGGGTTTAAAAACTATGCTTGAAGACGATAAATT
AAGAAAAGCGTATAAAAACAAAGCTAAAACTAGGGCTAAAGCCTTTGATAAAGTAAAAATTGCACGCGATGCTTT
GAAATATTTATTAGGATAAAAGATGTTGAAAAAAGAGTATTTAAAAAACCCTTATTTAGTTTTGTTTGCGATGAT
TGTATTAGCTTATGTTTTTAGTGTATTTTGCAGGTTTTATTGGGTTTGGTGGGCAAGTGAGTTTAACGAGTATTT
TTTCAATAATCAATTAATGATCATTTCAAACGATGGCTATGCTTTTGCTGAGGGCGCAAGAGATATGATAGCAGG
TTTTCATCAGCCTAATGATTTGAGTTATTATGGATCTTCTTTATCTACGCTTACTTATTGGCTTTATAAAATCAC
ACCTTTTTCTTTTGAAAGTATCATTTTATATATGAGTACTTTTTTATCTTCTTTGGTGGTGATTCCTATTATTTT
ACTAGCTAATGAATACAAACGCCCTTTAATGGGCTTTGTAGCTGCTCTTTTAGCAAGTGTAGCAAACAGTTATTA
TAATCGCACTATGAGTGGGTATTATGATACGGATATGCTGGTAATTGTTTTACCTATGTTTATTTTATTTTTTAT
GGTAAGAATGATTTTAAAAAAAGACTTTTTTTCATTGATTGCCTTGCCATTATTTATAGGAATTTATCTTTGGTG
GTATCCTTCAAGTTATACTTTAAATGTAGCTTTAATTGGACTTTTTTTAATTTATACACTTATTTTTCATAGAAA
AGAAAAGATTTTTTATATAGCTGTGATTTTGTCTTCTCTTACTCTTTCAAATATAGCATGGTTTTATCAAAGTGC
CATTATAGTAATACTTTTTGCTTTATTTGCTTTAGAGCAAAAACGCTTAAATTTTATGATTATAGGAATTTTAGG
TAGTGCAACTTTGATATTTTTGATTTTAAGTGGTGGGGTTGATCCCATACTTTATCAGCTTAAATTTTATATTTT
TAGAAGCGATGAAAGTGCGAATTTAACACAGGGCTTTATGTATTTTAATGTTAATCAAACCATACAAGAAGTTGA
AAATGTAGATTTTAGCGAATTTATGCGAAGAATTAGTGGTAGTGAAATTGTTTTCTTGTTTTCTTTGTTTGGTTT
TGTATGGCTTTTGAGAAAACATAAAAGTATGATTATGGCTTTACCTATATTGGTGCTTGGGTTTTTAGCCTTAAA
AGGAGGACTTAGATTTACCATTTATTCTGTACCTGTAATGGCTTTAGGATTTGGTTTTTTATTGAGCGAGTTTAA
GGCTATATTGGTTAAAAAATATAGCCAATTAACTTCAATGTTTGTATTGTTTTTGCAACTATTTTGACTTTGGCT
CCAGTATTTATCCATATTTACAACTATAAAGCGCCAACAGTTTTTTCTCAAAATGAAGCATCATTATTAAATCAA
TTAAAAAATATAGCCAATAGAGAAGATTATGTGGTAACTTGGTGGGATTATGGTTATCCTGTGCGTTATTATAGC
GATGTGAAAACTTTAGTAGATGGTGGAAAGCATTTAGGTAAGGATAATTTTTTCCCTTCTTTTTCTTTAAGTAAA
GATGAACAAGCTGCAGCTAATATGGCAAGACTTAGTGTAGAATATACAGAAAAAAGCTTTTATGCTCCGCAAAAT
GATATTTTAAAATCAGACATTTTACAAGCCATGATGAAAGATTATAATCAAAGCAATGTGGATTTATTTCTAGCT
TCATTATCAAAACCTGATTTTAAAATCGATACACCAAAAACTCGTGATATTTATCTTTATATGCCCGCTAGAATG
TCTTTGATTTTTTCTACGGTGGCTAGTTTTTCTTTTATTAATTTAGATACAGGAGTTTTGGATAAACCTTTTACC
TTTAGCACAGCTTATCCACTTGATGTTAAAAATGGAGAAATTTATCTTAGCAACGGAGTGGTTTTAAGCGATGAT
TTTAGAAGTTTTAAAATAGGTGATAATGTGGTTTCTGTAAATAGTATCGTAGAGATTAATTCTATTAAACAAGGT
GAATACAAAATCACTCCAATCGATGATAAGGCTCAGTTTTATATTTTTTATTTAAAGGATAGTGCTATTCCTTAC
GCACAATTTATTTTAATGGATAAAACCATGTTTAATAGTGCTTATGTGCAAATGTTTTTTTTGGGAAATTATGAT
AAGAATTTATTTGACTTGGTGATTAATTCTAGAGATGCTAAAGTTTTTAAACTTAAAATTTAAGGGTTGAAAATG
AGAATAGGATTTTTATCACATGCAGGAGCGAGTATTTATCATTTTAGAATGCCTATTATAAAAGCGTTAAAAGAT
AGAAAAGACGAAGTTTTTGTTATAGTGCCGCAAGATGAATACACGCAAAAACTTAGAGATCTTGGCTTAAAAGTA
ATTGTTTATGAGTTTTCAAGAGCTAGTTTAAATCCTTTTGTGGTTTTAAAGAATTTTTTTTATCTTGCTAAGGTT
TTGAAAAATTTAAATCTTGATTTTATTCAAAGTGCGGCACACAAAAGCAATACTTTTGGAATTTTAGCAGCAAAA
TGGGCAAAAATTCCTTATCGTTTTGCCTTAGTAGAAGGCTTGGGATCTTTTTATATAGATCAAGGTTTTAAGGCA
AATTTAGTGCGTTTTGTTATTAATAGTCTTTATAAATTAAGTTTTAAATTTGCACACCAATTTATTTTTGTCAAT
GAAAGTAATGCTGAGTTTATGCGGAATTTAGGACTTAAAGAAAATAAAATTTGCGTGATAAAATCTGTAGGGATC
AATTTAAAAAAATTTTTTCCTATTTATGTAGAATCGGAAAAAAAAGAGCTTTTTTGGAAAAATTTAAACATAGAT
AAAAAACCCATTGTGCTTATGATAGCAAGAGCTTTATGGCATAAGGGTGTAAAAGAATTTTATGAAAGTGCTACT
ATGCTAAAAGACAAAGCAAATTTTGTTTTAGTTGGTGGAAGAGATGAAAATCCTTCTTGTGCAAGTTTGGAGTTT
TTAAACTCTGGCGCGGTGCATTATTTGGGTGCTAGAAGTGATATAGTCGAGCTTTTGCAAAATTGTGATATTTTT
GTTTTGCCAAGCTATAAAGAAGGCTTTCCTGTAAGTGTTTTGGAGGCAAAAGCTTGCGGTAAGGCTATAGTGGTG
AGTGATTGTGAAGGTTGTGTGGAGGCTATTTCTAATGCTTATGATGGACTTTGGGCAAAAACAAAAAATGCTAAA

GATTTAAGCGAAAAAATTTCACTTTTATTAGAAGATGAAAAATTAAGATTAAATTTAGCTAAAAATGCCGCCCAA
GATGCTTTACAATACGATGAAAATATAATCGCACAGCGTTATTTAAAACTTTATGATAGGGTAATTAAGAATGTA
TGAAAAAGTTTTTAAAAGAATTTTTGATTTTATTTTAGCTTTAGTGCTTTTAGTGCTTTTTTCTCCGGTGATTTT
AATCACTGCTTTACTTTTAAAAATCACTCAAGGAAGTGTGATTTTTACCCAAAATCGTCCCGGGTTAGATGAAAA
AATTTTTAAAATTTATAAATTTAAAACCATGAGCGATGAAAGAGATGAAAAGGGTGAGTTATTAAGCGATGAATT
GCGTTTGAAAGCTTTTGGAAAAATCGTTAGAAGCTTAAGTTTGGATGAGCTTTTGCAACTTTTTAATGTTTTAAA
AGGGGATATGAGTTTTGTTGGACCTAGACCTCTTTTGGTTGAGTATTTGCCTCTTTACAATAAAGAGCAAAAATT
GCGTCATAAAGTGCGTCCAGGTATAACAGGATGGGCGCAGGTAAATGGTAGAAATGCTATTTCTTGGCAGAAAAA
ATTCGAACTTGATGTGTATTATGTGAAAAATATTTCTTTTTTGCTTGATTTAAAAATCATGTTTTTAACAGCTTT
AAAGGTTTTAAAACGAAGTGGGGTAAGCAAAGAAGGCCATGTTACAACAGAGAAATTTAATGGCAAGAACTGAAA
AAATTTATATTTATGGTGCTAGTGGTCATGGGCTTGTTTGTGAAGATGTGGCTAAAAATATGGGTTATAAAGAAT
GTATTTTTTTAGATGATTTTAAAGGAATGAAATTTGAAAGTACCTTACCTAAATATGATTTTTTTATAGCCATAG
GAAACAATGAAATTCGAAAAAAGATTTATCAAAAAATTTCAGAAAATGGCTTTAAAATTGTCAATCTTATCCATA
AAAGCGCGCTTATAAGTCCTAGCGCAATCGTGGAAGAAAATGCAGGAATTTTAATCATGCCTTATGTAGTGATTA
ACGCTAAAGCTAAAATAGAAAAAGGTGTGATTTTAAATACTTCAAGCGTAATTGAGCATGAATGTGTGATAGGGG
AATTTTCTCATGTGAGTGTGGGAGCTAAATGTGCGGGTAATGTAAAAATTGGTAAAAATTGTTTTTTAGGGATTA
ATTCTTGTGTTTTGCCTAATTTAAGTTTGGCAGATGATAGTATTTTAGGTGGTGGAGCAACTTTAGTTAAAAATC
AAGATGAAAAAGGTGTTTTTGTGGGAGTACCTGCAAAAAGGATGTAAATTGCATTTTAATAACAATCTTGTTGTT
CACTATATAGTAAATCCTTCGCCTTTGGGGTGGATTGTCATTAATTTACTAACCATATGTCTAATATGCTACATA
TTTCCTTTGAAAAATTCTTTAAAACACAAAAAACTTTTTAGTCTTAAAGCAAATGTAAATTCTAAAAATAGTAGG
ATTATAAAATATACAGGTATTGCTGCTTTTTTGGGTGGATTAATAGGAATTTGGTATAATTTTGAAGGTTTTTAT
CAACTTCTTTTTTTCTTTGAATTAGAAAATGAAAATTTAAAAACACTTTGGAGTTTGCAAGTATCAGTTTCTTCT
GTGATAACAGGTATGTTATTATTGTTGATATATGTTATAAATTTAGCAATGGTTTGTGAAAATGGAATTTATATA
GTTAGTAAATTTAATCTTTTTTATATGTATTTTATAAAACGAGAAGATTTGGAAATTGTTAAAATAGAAAAAATG
AAATTTTTAAATCAAGTTGAAGTTTGTTTTGTTATCAAAACAAAAAATAAAATACTCCTTAAATGCTTTGAAAGT
ATTTATAAAAAAGAAGACTTAGAAAAGCTTAAAAATTGGTATGAAAACAAGCTTTGACTATAAAAAGAATTTAAA
TATTTGAATCTTTGTAAATTTTTTTAGGTAAAATAGAGTCAATTTATAAAAATTTTGTTTTACACAAAGGATAAA
TCATGAGATTTTTTCTTTCTCCTCCGCATATGGGTGGTAATGAATTAAAATACATAGAAGAAGTTTTCAAAAGCA
ATTATATAGCACCTTTGGGTGAATTTGTAAATCGCTTTGAACAAAGTGTCAAGGCTTACAGTAAAAGTGAAAATG
CCTTAGCTTTAAATTCAGCCACAGCGGCTTTGCATTTAGCTTTAAGGGTGGCAGGGGTAAAACAAGATGATATTG
TTTTGGCTTCTTCTTTTACTTTTATCGCTTCAGTAGCACCTATTTGTTATCTTAAAGCAAAACCTGTATTTATAG
ATTGTGATGAAACTTATAATATCGATGTAGATTTATTAAAGCTTGCTATTAAAGAATGTGAAAAAAAACCAAAAG
CATTGATTTTAACTCATCTTTATGGCAATGCGGCTAAAATGGATGAAATTGTTGAAATTTGCAAAGAAAATGAAA
TTGTTTTAATCGAAGATGCTGCTGAAGCTTTAGGAAGTTTTTATAAGAATAAAGCTTTAGGAACTTTTGGAGAAT
TTGGAGCTTATTCTTATAATGGCAATAAAATTATCACCACTTCAGGTGGAGGTATGCTTATAGGAAAAAATAAAG
AAAAGATTGAAAAAGCAAGATTTTATAGCACTCAAGCTAGGGAAAATTGTTTGCATTATGAACATTTAGATTATG
GTTATAATTACCGCTTAAGCAATGTTTTAGGAGCTATTGGCGTAGCGCAAATGGAGGTTTTAGAACAAAGAGTGC
TTAAAAAAAGAGAAATTTATGAGTGGTATAAAGAATTTTTAGGAGAGTGTTTTAGCTTTTTAGATGAATTAGAAA
ATTCAAGAAGCAATCGCTGGTTAAGTACAGCTTTGATTGATTTTGATAAAAATGAACTTAATTCTTGTCAAAAAG
ATATAAATATCAGTCAAAAAAATATTACTTTGCATCCAAAAATTTCAAAACTCATAGAAGATTTGAAAAATGAAC
AAATAGAAACAAGACCATTATGGAAAGCTATGCACGCTCAAGAAGTATTTAAAGGAGCTAAGGCTTATCTTAATG
GCAATAGTGAGTTATTTTTCCAAAAAGGAATTTGTTTGCCAAGTGGCACGGCGATGAGTAAAGATGATGTTTATG
AAATTTCAAAACTGATCTTAAAGAGCATAAAGGCTTAAAATGATTTTTTATAAAAGCAAAAGATTAGCATTTTTT
TTAACTTCAGATATTGTTTTAATTTTACTTAGCGTTTATCTGGCTTTTTCTTTGAGATTTAGTGGAGATATTCCG
AGTATTTTTTATCATGGTATGATGGTTTCTGCTATTATTTTGCTTGTTTTAAAACTTTCATTTTTGTTTGTTTTT
AGAATTTATAAAGTAGCTTGGAGATTTTTTTCTCTCAATGAAGCAAGAAAGATTTTTATCGCTTTGCTTTTAGCT
GAGTTTTGTTTTTTTCTTATTTTTTATTTTTTTAGTGATTTTTTTAATCCTTTTCCAAGAAGTGCTATTGTGATA
GATTTTGTTCTTTCTTATATGTTTATAGGTACTTTAAGAATTAGCAAAAGAATGCTTGTGGATTTTAAACCTTCT
AGAATGAAAGAAGAAGAAACTCCTTGTATTGTAGTAGGGGCAACTTCTAAGGCTTTGCATTTGTTAAAAGGTGCA
AAAGAAGGTTCTTTAGGGCTTTTTCCTGTAGGCGTAGTTGATGCGAGAAAAGAGCTTATAGGGACTTATTGTGAT
AAATTTATTGTAGAAGAAAAAGAAAAAATAAAATCTTATGTAGAACAAGGGGTAAAAACTGCCATTATTGCTTTA
AGACTTGAACAAGAAGAGCTTAAAAAACTTTTTGAAGAACTTGTAGCTTATGGTATTTGCGATGTAAAAATATTT
TCTTTTACAAGAAACGAAGCAAGAGATATCAGTATAGAAGACTTGCTTGCTAGAAAACCAAAAGATTTAGATGAT
AGTGCTGTGGCGGCTTTTTTAAAAGATAAGGTAGTTTTGGTAAGTGGAGCAGGTGGAACTATAGGCAGTGAACTT
TGTAAGCAATGTATTAAATTTGGTGCTAAGCATCTTATCATGGTTGATCATAGTGAGTATAATCTTTATAAGATC
AATGATGATTTAAATTTATATAAAGAAAAAATTACTCCTATTTTACTGAGTATTTTAGATAAGCAAAGTTTAGAT
GAGGTATTAAAAACTTATAAACCCGAGCTTATTTTACATGCAGCCGCTTATAAACATGTGCCTCTTTGCGAACAA
AATCCACATTCAGCAGTAATCAATAATATTTTAGGAACTAAAATTTTATGCGACAGTGCTAAAGAAAACAAAGTA
GCTAAATTTGTGATGATAAGTACAGATAAAGCAGTACGACCAACAAATATTATGGGTTGCACTAAGAGAGTTTGC
GAGCTTTATACTTTAAGTATGAGTGATGAAAATTTTGAAGTTGCTTGTGTGCGTTTTGGTAATGTTTTAGGTTCT

AGTGGTAGTGTGATACCGAAATTTAAAGCACAAATTGCCAATAATGAGCCTTTAACTTTAACGCACCCTGATATA
GTGCGTTATTTTATGCTTGTGGCTGAGGCAGTGCAACTTGTTTTACAAGCTGGAGCTATCGCAAAAGGGGGAGA
ACTTTTTGTTTTGGATATGGGTAAGCCTGTGAAAATCATAGATTTAGCTAAAAAAATGCTTTTACTTTCTAATCG
CAATGATTTAGAAATTAAAATCACAGGCTTAAGAAAAGGTGAGAAGCTTTATGAAGAGCTTTTGATTGATGAAAA
TGATGCTAAAACACAATATGAGAGTATTTTTGTAGCAAAGAATGAGAAGGTTGACCTTGATTGGCTTAATAAAGA
GATAGAAAATTTACAAATATGTGAAGATATTTCAGAGGCTTTATTAAAGATTGTACCTGAATTTAAACACAATAA
AGAAGGTGTATAATGTATATAAAAGATATACAAAGATTTGAAGATAATCGCTATCGTGCTAGAGCTTATATGAGT
TATATTTTAACAAGAAATCTGCCCAATAAACTTCCTGACATTCACCTTGAAACGATTAAAACAGCTTTGGATAAA
ATAGCTCATGAAGTTGTTGTTTTTGATGCTTTGTATATTTTAGATATTTCAGGCATGCAAATAGAAAATGCGATT
TCCTTAAATAAAGCTCATGAAATAGGGCAGGGTGAGGATAGAAGTACTCGTTCTTATTTTTATAGAGCTGTAAAA
TTAAGACGATGTGTTTTGAGCGATCCTTATCCTTCGGTTTTAAATAATGAGCTTTGCGTGACAGCTTCTATGCCA
ATTTACGATGATAAAAATAACTTGCTTTTTGTTGTTTGTATTGATATCAAGCTTGAAGATATTTTAAAGATTATT
CAAGCAGGAAAATTTGAATTTGTTTTTACTCAGTTTAGTCGTTTGGTATATTTTTGCTTCGCACTGGTTTTATTT
GTGATTACTTGTTTTTTATTTCAAAAAGGTTTTTTTAGTCTTTTTGATAATCAAGCTATAGGTATAGAACATATG
TTTGAAAGTACCATCGCTATAACTTTGGCTTTAGCTATTTTTGATTTGGCAAAAACTTTGATCGAACAAGAAGTA
TTAGGAAGGACGAAAAAAGAAGAAGGTGGAATTCAAAAAACTATGGTGAGATTTTTGGGTTCTATTATCATTGCT
TTAGCTATAGAAGCTTTGATGTTGGTATTTAAACTTGCTATTGGTGATCTTTCTCAGATGATTTATGCGATTTAT
CTTATCGGTGGAGTGAGCTTGCTTCTTTTAGGTTTAAGTGTATATTTATTTACGGTTAAGTATAAAAATAATAAT
ATTTGAACTTAGT (SEQ ID NO: 42).

[00218] In some embodiments, the nucleic acid comprises a *C. jejuni pgl* operon (provided as SEQ ID NO: 42). In some embodiments, the nucleic acid comprises a *C. jejuni pgl* operon, wherein the *C. jejuni pgl* operon comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 42. In some embodiments, the nucleic acid comprises *C. jejuni pgl* operon, wherein *C. jejuni pgl* operon comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 42.

[00219] *wlaA* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *wlaA* gene (*e.g.*, a *C. jejuni wlaA* gene).

[00220] In some embodiments, the nucleic acid comprising a *wlaA* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *wlaA* gene is located on a chromosome of the bacterium.

[00221] The nucleic acid sequence of an exemplary *C. jejuni wlaA* gene is provided below:

ATGGCAAAAAATGAAGGTTATATTTGTGTTTTTGATTGTGAGAGTGTGCCAGATGTTGAGCT
TATCCGCAAAACTTTGGGTTTTGAAGGAAGTGATTTAGAGGTAAGTTTAAAAGCACTTCAGT
GGCAAAAAGAACAAAGTGGGAGTGAGTTTTTGCCTTTGCCTTATCATAAAATTATCAGTATT
TGTGCGGTTTTAAGTGATAATTTTGGAAAATTTATCAAAGTGAATAAAATTGATGGACAAAA

TGAAAAAGAAATGATTGAGAATTTTTTCAATTTTATAGAAAATTATGAGCCAAAATTAGTCA
GTTTTAATGGTAAAAATTTCGATATGCCTGTTCTTGTTTTAAGGGCTTTAAAATACAATTTA
AAAGCAGCAACTTATTTGGATACTCAAAGTGATAAATGGAATAATTATAAAACAAGATTTTC
AGAATTAAAACATTGTGATTTATTAGAATCCTTAGGATCTAACGGGCGTGGAATAAAGCTTG
ATACACTTTGTTCTATGGTGGGTTTGCCAGGAAAATATGATGTGCATGGCGATGAGGTAATG
AAACTTTTTTATGAAAATAAACTTGAAAAAATCCACGAATATTGTGAAAGTGATGTTTTAAA
CACCTATATGCTTTTTTTAAAATATGAACTTATTAAAGCTAATGTTGATGAAGAAGATTATG
TTGGTTTTCTTTCTTATATGAGAGATTTCTTGTGTGCAAAAAAATCAGATCGTTCTTATACA
GAAGTTTTTGCAAAAGCTTGTGAGAGTGAAATTTCAAAAGTTCGATCTTAA (SEQ ID NO: 43)

[00222] The amino acid sequence of the WlaA protein encoded by the nucleic acid of SEQ ID NO: 43 is provided below:

MAKNEGYICVFDCESVPDVELIRKTLGFEGSDLEVSLKALQWQKEQSGSEFLPLPYHKIISI
CAVLSDNFGKFIKVNKIDGQNEKEMIENFFNFIENYEPKLVSFNGKNFDMPVLVLRALKYNL
KAATYLDTQSDKWNNYKTRFSELKHCDLLESLGSNGRGIKLDTLCSMVGLPGKYDVHGDEVM
KLFYENKLEKIHEYCESDVLNTYMLFLKYELIKANVDEEDYVGFLSYMRDFLCAKKSDRSYT
EVFAKACESEISKVRS

[00223] In some embodiments, the nucleic acid comprises a *C. jejuni wlaA* gene (provided as SEQ ID NO: 43). In some embodiments, the nucleic acid comprises a *wlaA* gene, wherein the *wlaA* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO:43. In some embodiments, the nucleic acid comprises a *wlaA*gene, wherein the *wlaA* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 43.

[00224] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a WlaA protein, wherein said WlaA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 44. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a WlaA protein, wherein said WlaA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 44.

[00225] *wlaJ* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *wlaJ* gene (*e.g.*, a *C. jejuni wlaA* gene).

[00226] In some embodiments, the nucleic acid comprising a *wlaJ* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *wlaJ* gene is located on a chromosome of the bacterium.

[00227] The nucleic acid sequence of an exemplary *C. jejuni wlaJ* gene is provided below:

ATGAAAAAGGTGTTTTTGTGGGAGTACCTGCAAAAAGGATGTAAATTGCATTTTAATAACAA
TCTTGTTGTTCACTATATAGTAAATCCTTCGCCTTTGGGGTGGATTGTCATTAATTTACTAA
CCATATGTCTAATATGCTACATATTTCCTTTGAAAAATTCTTTAAAACACAAAAAACTTTTT
AGTCTTAAAGCAAATGTAAATTCTAAAAATAGTAGGATTATAAAATATACAGGTATTGCTGC
TTTTTTGGGTGGATTAATAGGAATTTGGTATAATTTTGAAGGTTTTTATCAACTTCTTTTTT
TCTTTGAATTAGAAAATGAAAATTTAAAAACACTTTGGAGTTTGCAAGTATCAGTTTCTTCT
GTGATAACAGGTATGTTATTATTGTTGATATATGTTATAAATTTAGCAATGGTTTGTGAAAA
TGGAATTTATATAGTTAGTAAATTTAATCTTTTTTATATGTATTTTATAAAACGAGAAGATT
TGGAAATTGTTAAAATAGAAAAAATGAAATTTTTAAATCAAGTTGAAGTTTGTTTTGTTATC
AAAACAAAAAATAAAATACTCCTTAAATGCTTTGAAAGTATTTATAAAAAAGAAGACTTAGA
AAAGCTTAAAAATTGGTATGAAAACAAGCTTTGA (SEQ ID NO: 45).

[00228] The amino acid sequence of the WlaJ protein encoded by the nucleic acid of SEQ ID NO: 45 is provided below:

MKKVFLWEYLQKGCKLHFNNNLVVHYIVNPSPLGWIVINLLTICLICYIFPLKNSLKHKKLFSLKANV
NSKNSRIIKYTGIAAFLGGLIGIWYNFEGFYQLLFFFELENENLKTLWSLQVSVSSVITGMLLLLIYV
INLAMVCENGIYIVSKFNLFYMYFIKREDLEIVKIEKMKFLNQVEVCFVIKTKNKILLKCFESIYKKE
DLEKLKNWYENKL.

[00229] In some embodiments, the nucleic acid comprises a *C. jejuni wlaJ* gene (provided as SEQ ID NO: 45). In some embodiments, the nucleic acid comprises a *wlaJ* gene, wherein the *wlaJ* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 45. In some embodiments, the nucleic acid comprises a *wlaJ* gene, wherein the *wlaJ* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 45.

[00230] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a WlaJ protein, wherein said WlaJ protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 46. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a WlaJ protein, wherein said WlaJ protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 46.

[00231] *gne* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *gne* gene (*e.g.*, a *C. jejuni gne* gene).

[00232] In some embodiments, the nucleic acid comprising a *gne* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *gne* gene is located on a chromosome of the bacterium.

[00233] The nucleic acid sequence of an exemplary *C. jejuni gne* gene is provided below:

ATGATGATGAAAATTCTTATTAGCGGTGGTGCAGGTTATATAGGTTCTCATACTTTAAGACAATTTTTAAAAACA
GATCATGAAATTTGTGTTTTAGATAATCTTTCTAAGGGTTCTAAAATCGCAATAGAAGATTTGCAAAAAACAAGA
GCTTTTAAATTTTTCGAACAAGATTTAAGTGATTTTCAAGGCGTAAAAGCATTGTTTGAGAGAGAAAAATTTGAC
GCTATTGTGCATTTTGCAGCAAGCATTGAAGTTTTTGAAAGTATGCAAAATCCTTTAAAATATTATATGAACAAC
ACTGTTAATACGACAAATCTCATCGAAACTTGTTTGCAAACTGGAGTGAATAAATTTATATTTTCTTCAACGGCG
GCCACTTATGGCGAACCACAAACTCCCGTTGTGAGCGAAACAAGTCCTTTAGCACCTATTAATCCTTATGGGCGT
AGTAAGCTTATGAGTGAAGAAGTTTTGCGTGATGCAAGTATGGCAAATCCTGAATTTAAGCATTGTATTTTAAGA
TATTTTAATGTTGCAGGTGCTTGTATGGATTATACTTTAGGACAACGCTATCCAAAAGCGACTTTGCTTATAAAA
GTTGCAGCTGAATGTGCCGCAGGAAAACGTGATAAACTTTTCATATTTGGCGATGATTATGATACAAAAGATGGT
ACTTGCATAAGAGATTTTATCCATGTAGATGATATTTCAAGTGCACATTTAGCGGCTTTGGATTATTTAAAAGAG
AATGAAAGCAATGTTTTTAATGTAGGTTATGGACATGGTTTTAGCGTAAAAGAAGTGATTGAAGCGATGAAAAAA
GTTAGCGGAGTGGATTTTAAAGTAGAACTTGCCCCACGCCGTGCGGGTGATCCTAGTGTATTGATTTCTGATGCA
AGTAAAATCAGAAATCTTACTTCTTGGCAGCCTAAATATGATGATTTAGAGCTTATTTGTAAATCTGCTTTTGAT
TGGGAAAAACAGTGTTAA(SEQ ID NO: 47)

[00234] The amino acid sequence of the Gne protein encoded by the nucleic acid of SEQ ID NO: 47 is provided below:

MMMKILISGGAGYIGSHTLRQFLKTDHEICVLDNLSKGSKIAIEDLQKTRAFKFFEQD LSDFQGVKALFEREKFDAIVHFAASIEVFESMQNPLKYYMNNTVNTTNLIETCLQTG VNKFIFSSTAATYGEPQTPVVSETSPLAPINPYGRSKLMSEEVLRDASMANPEFKHCIL RYFNVAGACMDYTLGQRYPKATLLIKVAAECAAGKRDKLFIFGDDYDTKDGTCIRD FIHVDDISSAHLAALDYLKENESNVFNVGYGHGFSVKEVIEAMKKVSGVDFKVELAP RRAGDPSVLISDASKIRNLTSWQPKYDDLELICKSAFDWEKQC (SEQ ID NO: 48).

[00235] In some embodiments, the nucleic acid comprises a *C. jejuni gne* gene (provided as SEQ ID NO: 47). In some embodiments, the nucleic acid comprises a *gne* gene, wherein the *gne* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 47. In some embodiments, the nucleic acid comprises a *gne* gene, wherein the *gne* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 47.

[00236] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Gne protein, wherein said Gne protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 48. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a Gne protein, wherein said Gne protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 48.

[00237] *pglK* – In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglK* gene (*e.g.*, a *C. jejuni pglK* gene).

[00238] In some embodiments, the nucleic acid comprising a *pglK* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglK* gene is located on a chromosome of the bacterium.

[00239] The nucleic acid sequence of an exemplary *C. jejuni pglK* gene is provided below:

ATGCCTTTTATCACTTTGGCTAGTGATTTTTCTTATTTTGATCGTAATAAATATTTAATCMAGCCTAAAAGAATA
TCTTAATATCCCTGTTTTTGAAATCATTGTTTATTTTGGAGTGGGGCTTATTGTTTTTTATGTGTTTAGAGCTTT
GTTAAATGCGTATTATTTTCATCTTTTGGCALAGATTTTCTAAAGGGCGTTATCATGCGATCGCTTATAAGGTTT
TTTCTAAATTTTTAAATATTAATTATGAAAAATTTACTCAAAAAAATCAATCTGAAATTTTAAAGTCCATTACAG
GGIGAAGTTTATAATCTAAGCACTATGATTTCATCATTTTTACTTTTGATGAGTGAAATTTTTGTAGTACTTTTG
CTTTATGCTTTAATGCTTTTGATTAATTATAAAATCACTTTGTTTTTAVAGTATTTTTATGGTGTTAAATGCCTT
TATTTTAGTGAAAATTTTAAGCCCTATCATTAAAAAAGCAGGAGTAAGACGCGAAGAAGCGATGAAAAATTTCTT
TGAAATTTTAAATACAAATKTTAAATAATTTCAAATTTATTAAGCTTAAAACCAAAGAAGATGGAGTATTAAGTC
TTTTTAAAGCGCAAAGTGAAGCTTTTTCTAAAGCAAATATTACCAACGAAAGCGTAGCTGCGGTGKCCTAGAATT
TATCTTGAAGGAATAGGCTTTTGCGTACTTGTTTTTATCGTGGTATTTTTGGTTTTGAAAAATGAAAGTGATATT
TCAGGTATTTTATCCACGATTTCTATTTTTGTTTTAVGCGCTTTATCGCTTAATGCCAAGTGCAAATCGTATTAT
TACAAGTTATCATGATTTGCTTTATTATCATTCTTCTTTGGATATTATTTATCAAAATTTAAGACAAGAAGAAGA
AAATTTGYGGCGAGGAAAAATTAAGCTTTAATCAAGAGCTTAAAATTTGCAATCTTAGCTTTGGTTATGAGGGAA
AAAAATATTTATTTAAAAATCTTAACTTAAATATTAAAAAAGGCGAAAAAATCEGCTTTTATAGGGGAGAGTGGT
TGTGGAAAAAGTACCTTAGTAGATCTTATCATAGGACTTTTAAAACCAAAAGAAGGGCAAATTTTAATTGATGAG
CAAGAATTAAATGCAAATAATACALAAAAATTATCGCCAAAAAATAGGCTATATCCCGCAAAATATCTATCTTTT
TAATGACAGTATAGCTAAAAATATCACTTTTGGAGATGCGGTTGATGAAGAAAAACTTAATAGGGTTATCIAAAC
AAGCAAATTTAGAGCATTTTATAAAAAATTTACCTCAAGGAGTGCAAACAAAAGTGGGCGATGGGGGGAGTAATT
TAAGCGGGGGACAAAAACAACGCATAGCTATAGCAAGAGCTGTTATATTTAGAGCCTGAAATGTTAGTGCTTGAT
GAAGCAACTTCTGCGCTTGATACTCAAAGTGAAGCAAAAATTATGGATGAAATTTATAAAATTTCTAAAGATAAA
ACCATGATTATTSATCGCACATCGCCTTTCTACGATAACACAATGTGATAAGGTTTATCGTTTAGAACACGGTAA
GCTTAAAGAGGAGAAATGA (SEQ ID NO: 49)

[00240] The amino acid sequence of the PglK protein encoded by the nucleic acid of SEQ ID NO: 49 is provided below:

MPFITLASDFSYFDRNKYLISLKEYLNIPVFEIIVYFGVGLIVFYVFRALLNAYYFHLLARFSKGRYHAIAYKVF
SKFLNINYEKFTQKNQSEILKSITGEVYNLSTMISSFLLLMSEIFVVLLLYALMLLINYKITLFLSIFMVLNAFI
LVKILSPIIKKAGVRREEAMKNFFEILNTNLNNFKFIKLKTKEDGVLSLFKAQSEAFSKANITNESVAAVPRIYL
EGIGFCVLVFIVVFLVLKNESDISGILSTISIFVLALYRLMPSANRIITSYHDLLYYHSSLDIIYQNLRQEEENL
GEEKLSFNQELKICNLSFGYEGKKYLFKNLNLNIKKGEKIAFIGESGCGKSTLVDLIIGLLKPKEGQILIDEQEL
NANNTKNYRQKIGYIPQNIYLFNDSIAKNITFGDAVDEEKLNRVIKQANLEHFIKNLPQGVQTKVGDGGSNLSGG
QKQRIAIARALYLEPEMLVLDEATSALDTQSEAKIMDEIYKISKDKTMIIIAHRLSTITQCDKVYRLEHGKLKEE
K (SEQ ID NO: 50).

[00241] In some embodiments, the nucleic acid comprises a *C. jejuni pglK* gene (provided as SEQ ID NO: 49). In some embodiments, the nucleic acid comprises a *pglK* gene, wherein the *pglK* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 49. In some embodiments, the nucleic acid comprises a *pglK* gene, wherein the *pglK* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%,

at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 49.

[00242] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglK protein, wherein said PglK protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 50. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglK protein, wherein said PglK protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 50.

[00243] *pglH* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglH* gene (*e.g.*, a *C. jejuni pglH* gene).

[00244] In some embodiments, the nucleic acid comprising a *pglH* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglH* gene is located on a chromosome of the bacterium.

[00245] The nucleic acid sequence of an exemplary *C. jejuni pglH* gene is provided below:

AAAATAAGCTTTATTATCGCAACTTTAAATTCAGGAGGTGCTGAGCGTGCTTTAG
TAACCTTAGCTAATGCACTTTGCAAAGAGCATGAAGTAAGTATTATTAAATTTCA
TGCAGGAGAATCTTTTTATAAGCTTGAAAATGAAGTTAAAGTTACAAGTTTGGAA
CAATTTAGATTTGACACGCTTTATCATAAAATCGCAAGTCGTTTTAAGAAATTTTT
TGCTTTAAGAAAGGCTTTGAAAGAAAGTAAGTCTGATGTTTTTATTTCTTTTTTGG
ATACGACTAATATTGCTTGTATTGCTGCGAAAATAGGGCTTAAAACTCCACTCAT
TATAAGTGAGCATAGCAATGAAGCGTATTTAAAACCTAAAATTTGGCGTTTTTTA
AGAAGGGTAAGCTATCCTTTTTGTGATGCTTTAAGTGTGCTTGGAAGCAGTGATA
AGGTGTATTATGAAAGATTTGTAAAAAGGGTTAAGCTTTTATTAAACCCTTGTCA
TTTTAGCGATGAAATTTCTTTTGATTCTAGTTTTGAAAAGGAAAATTTGGTTCTTT
TTATAGGGCGTTTAGATCACAACAAAAACCCTGTAATGTTTTTAAAAGCTATAGC

GCATTTGGATAAAAATTTACAAGAAAATTATAAATTTGTTATAGCAGGAGATGG
ACAGTTAAGACAAGAACTTGAATATAAGGTAAAATCTTTAGGAATAAAAGTTGA
TTTTTTAGGACGCGTTGAAAATGTCAAGGCTCTTTATGAAAAAGCAAAAGTGCTT
TGCCTTTGTTCTTTTGTAGAGGGTTTGCCAACGGTTTTAATTGAAAGTTTGTATTT
TGAGGTTTGTAGAATTTCAAGTTCTTATTATAATGGTGCTAAGGATTTAATCAAA
GATAATCATGATGGGCTTTTGGTAGGTTGTGATGATGAAATAGCACTTGCTAAAA
AACTTGAACTTGTTTTAAATGATGAAAATTTTAGAAAAGAACTTGTAAATAATGC
CAAACAAAGGTGTAAAGACTTTGAAATTTCTCATATCAAAGAAGAATGGCTTAA
GCTTATAGCCGAGGTT (SEQ ID NO: 51)

[00246] The amino acid sequence of the PglH protein encoded by the nucleic acid of SEQ ID NO: 51 is provided below:

KISFIIATLNSGGAERALVTLANALCKEHEVSIIKFHAGESFYKLENEVKVTSLEQFRF
DTLYHKIASRFKKFFALRKALKESKSDVFISFLDTTNIACIAAKIGLKTPLIISEHSNEA
YLKPKIWRFLRRVSYPFCDALSVLGSSDKVYYERFVKRVKLLLNPCHFSDEISFDSSF
EKENLVLFIGRLDHNKNPVMFLKAIAHLDKNLQENYKFVIAGDGQLRQELEYKVKS
LGIKVDF
LGRVENVKALYEKAKVLCLCSFVEGLPTVLIESLYFEVCRISSSYYNGAKDLIKDNHD
GLLVGCDDEIALAKKLELVLNDENFRKELVNNAKQRCKDFEISHIKEEWLKLIAEV
(SEQ ID NO: 52)

[00247] In some embodiments, the nucleic acid comprises a *C. jejuni pglH* gene (provided as SEQ ID NO: 51). In some embodiments, the nucleic acid comprises a *pglH* gene, wherein the *pglH* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 51. In some embodiments, the nucleic acid comprises a *pglH* gene, wherein the *pglH* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 51.

[00248] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglH protein, wherein said PglH protein comprises an amino acid

sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 52. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglH protein, wherein said PglH protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 52.

[00249] *pglI* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglI* gene (*e.g.*, a *C. jejuni pglI* gene).

[00250] In some embodiments, the nucleic acid comprising a *pglI* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglI* gene is located on a chromosome of the bacterium.

[00251] The nucleic acid sequence of an exemplary *C. jejuni pglI* gene is provided below:

ATGCCTAAACTTTCTGTTATAGTACCAACTTTTAATCGTCAAGTTTTGTTAGAAAA
GGCTATTAAAAGCATACAAAATCAAGATTTTAAAGATTTAGAAATTATTGTAAGC
GATGATAATTCTAGCGATGATACTAAAAGTGTGGTGCAAAATTTACAAAAAGAT
GATGATCGCATTAAGTATTTTTTAAATCAAAATTACAAACAAGGTCCAAATGGCA
ATAAAAACAATGGCTTAGATCAAGCAAGTGGCGAGTTTGTAACTTTTTTAGATGA
TGATGATGAGCTTTTATCCGGGGCTTTAAGTACCTTGATGCAAAAAGCAAATGAG
GGTTATGCTCATGTTTTTGGAAATTGTTTGATAGAAAAAGAAGGAAATTTAAGCA
AGGAATTTAGCGGCAAGGGCTTGGAAAAAGATAGTGAAATTTCTAAAAAAGATT
TTTTAATGGCTAAATTTAGCGGAGAGTTTTTTTCTGTTTTTAAAAAATCCCTACTT
GAAAATAAGCGTTTTAATGAAGAATTTTATGGCAATGAAGCCACGCTTTGGGTAA
ATTTATACAAAGAAAAAAGTTTTTATATCCATAAGGCTTTTAGGATTTATAGAAT
TTTTAGGCAAGATAGCGTGACTTTAGGGGCGAGTAAAAATGCTTATAGGGTGTAT
TTGGATATTTAGAGCTTGCTAAAATTTTAGAAAATGAACTTAGAATGAGTAAGGA
TAAAGATTATAAAAAAACTTGTGCGAGTTATTATAAAATGGCAGCTTATTATGCA
AAACTTGCAAAAAATTATAAAGCCCTTTATAAATGTTTGTTTAAAAGCCTAAGTA

TAAAAATCAACGCTCCTGCTTTGATATTACTCATTTTAAGTATAATTCCAAATAAT ATGATTGAAAAATTATCAAAAATTCGGGTG (SEQ ID NO: 53)

[00252] The amino acid sequence of the PglI protein encoded by the nucleic acid of SEQ ID NO: 53 is provided below:

MPKLSVIVPTFNRQVLLEKAIKSIQNQDFKDLEIIVSDDNSSDDTKSVVQNLQKDDDR IKYFLNQNYKQGPNGNKNNGLDQASGEFVTFLDDDDELLSGALSTLMQKANEGYA HVFGNCLIEKEGNLSKEFSGKGLEKDSEISKKDFLMAKFSGEFFSVFKKSLLENKRFN EEFYGNEATLWVNLYKEKSFYIHKAFRIYRIFRQDSVTLGASKNAYRVYLGYLELAK ILENELRMSKDKDYKKTCASYYKMAAYYAKLAKNYKALYKCLFKSLSIKINAPALIL LILSIIPNNMIEKLSKIRV (SEQ ID NO: 54)

[00253] In some embodiments, the nucleic acid comprises a *C. jejuni pglI* gene (provided as SEQ ID NO: 53). In some embodiments, the nucleic acid comprises a *pglI* gene, wherein the *pglI* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 53. In some embodiments, the nucleic acid comprises a *pglI* gene, wherein the *pglI* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 53.

[00254] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglI protein, wherein said PglI protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 54. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglI protein, wherein said PglI protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,

at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 54.

[00255] *pglJ* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglJ* gene (*e.g.*, a *C. jejuni pglJ* gene).

[00256] In some embodiments, the nucleic acid comprising a *pglJ* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglJ* gene is located on a chromosome of the bacterium.

[00257] The nucleic acid sequence of an exemplary *C. jejuni pglJ* gene is provided below:

TTAGGCATTTTTATTTATTCTTTAGGAAGTGGTGGTGCTGAAAGAGTTGTGGCGA
CTTTATTGCCTATTTTAAGTTTGAAATTTGAAGTGCATTTGATCTTGATGAATGAT
AAAATTTCTTATGAAATTCCAGAGTGTCAAATTCATTTTTTAGAATGTTCAAAAC
CTAGTGAAAATCCTATTTTGAAATTTTTAAAACTACCTTTTTTGGCTTTAAAATAC
AAAAAACTTTGCAGAAATTTAGGTATTGATACAGAATTTGTTTTTTTAAATCGAC
CTAATTATATAGCTTTAATGGCAAGAATGTTTGGAAACAAAACTCGCCTTGTGAT
CAATGAATGCACTACGCCAAGTGTGATGTATATGAAAAATAATTTTAATTCTTTG
GTAAATAAATTTTTAATTTCTTTGCTTTACCCAAAAGCTGATTTAATCTTGCCTAA
TTCTAAGGGAAATTTAGAAGATTTAGTGCAAAATTTTAGTATAAGTCCAAAAAAA
TGTGAAATTTTATACAATGCCATCGATTTAGAAAACATAGGGCAAAAAGCCCTTG
AAGACATAGCTTTAAAAGATAAATTTATTTTAAGTGTAGGCAGGCTTGATAAAGG
TAAAAATCATGCTTTATTAATTCGTGCTTATGCGAGATTGAAAACAGATTTAAAG
CTTGTGATTTTAGGTGAAGGTGTGCTTAAGGATGAGCTTTTAGCTTTGATTAAAG
AATTAAATTTGGAAGAAAAGGTTTTGCTTTTAGGATTTGATAATAATCCTTATAA
ATACATGGCTAAATGCGAATTTTTTGCTTTTGCTTCTGTGTTTGAAGGTTTTTCAA
ATGTTTTAATCGAAAGTTTGGCTTGTTCTTGTGCGGTGGTTTGCACTGATCATAAA
AGTGGTGCAAGAGAGCTTTTTGGCGATGATGAATTTGGACTTTTAGTAGAAGTAG
ATAATGAAAACTCTATGTTTCAGGGTTTAAAAACTATGCTTGAAGACGATAAATT
AAGAAAAGCGTATAAAAACAAAGCTAAAACTAGGGCTAAAGCCTTTGATAAAGT
AAAAATTGCACGCGATGCTTTGAAATATTTATTAGGATAA (SEQ ID NO: 55)

[00258] The amino acid sequence of the PglJ protein encoded by the nucleic acid of SEQ ID NO: 55 is provided below:

LGIFIYSLGSGGAERVVATLLPILSLKFEVHLILMNDKISYEIPECQIHFLECSKPSENPIL
KFLKLPFLALKYKKLCRNLGIDTEFVFLNRPNYIALMARMFGNKTRLVINECTTPSV

MYMKNNFNSLVNKFLISLLYPKADLILPNSKGNLEDLVQNFSISPKKCEILYNAIDLE NIGQKALEDIALKDKFILSVGRLDKGKNHALLIRAYARLKTDLKLVILGEGVLKDELL ALIKELNLEEKVLLLGFDNNPYKYMAKCEFFAFASVFEGFSNVLIESLACSCAVVCTD HKSGARELFGDDEFGLLVEVDNENSMFQGLKTMLEDDKLRKAYKNKAKTRAKAFD KVKIARDALKYLLG (SEQ ID NO: 56)

[00259] In some embodiments, the nucleic acid comprises a *C. jejuni pglJ* gene (provided as SEQ ID NO: 55). In some embodiments, the nucleic acid comprises a *pglJ* gene, wherein the *pglJ* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 55. In some embodiments, the nucleic acid comprises a *pglJ* gene, wherein the *pglJ* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 55.

[00260] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglJ protein, wherein said PglJ protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 56. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglJ protein, wherein said PglJ protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 56.

[00261] *pglB* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglB* gene (*e.g.*, a *C. jejuni pglB* gene).

[00262] In some embodiments, the nucleic acid comprising a *pglB* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglB* gene is located on a chromosome of the bacterium.

[00263] The nucleic acid sequence of an exemplary *C. jejuni pglB* gene is provided below:

ATGTTGAAAAAAGAGTATTTAAAAAACCCTTATTTAGTTTTGTTTGCGATGATTG
TATTAGCTTATGTTTTTAGTGTATTTTGCAGGTTTTATTGGGTTTGGTGGGCAAGT
GAGTTTAACGAGTATTTTTTCAATAATCAATTAATGATCATTTCAAACGATGGCT
ATGCTTTTGCTGAGGGCGCAAGAGATATGATAGCAGGTTTTCATCAGCCTAATGA
TTTGAGTTATTATGGATCTTCTTTATCTACGCTTACTTATTGGCTTTATAAAATCA
CACCTTTTTCTTTTGAAAGTATCATTTTATATATGAGTACTTTTTTATCTTCTTTGG
TGGTGATTCCTATTATTTTACTAGCTAATGAATACAAACGCCCTTTAATGGGCTTT
GTAGCTGCTCTTTTAGCAAGTGTAGCAAACAGTTATTATAATCGCACTATGAGTG
GGTATTATGATACGGATATGCTGGTAATTGTTTTACCTATGTTTATTTTATTTTTTA
TGGTAAGAATGATTTTAAAAAAAGACTTTTTTTCATTGATTGCCTTGCCATTATTT
ATAGGAATTTATCTTTGGTGGTATCCTTCAAGTTATACTTTAAATGTAGCTTTAAT
TGGACTTTTTTTAATTTATACACTTATTTTTCATAGAAAAGAAAAGATTTTTTATA
TAGCTGTGATTTTGTCTTCTCTTACTCTTTCAAATATAGCATGGTTTTATCAAAGT
GCCATTATAGTAATACTTTTTGCTTTATTTGCTTTAGAGCAAAAACGCTTAAATTT
TATGATTATAGGAATTTTAGGTAGTGCAACTTTGATATTTTTGATTTTAAGTGGTG
GGGTTGATCCCATACTTTATCAGCTTAAATTTTATATTTTTAGAAGCGATGAAAGT
GCGAATTTAACACAGGGCTTTATGTATTTTAATGTTAATCAAACCATACAAGAAG
TTGAAAATGTAGATTTTAGCGAATTTATGCGAAGAATTAGTGGTAGTGAAATTGT
TTTCTTGTTTTCTTTGTTTGGTTTTGTATGGCTTTTGAGAAAACATAAAAGTATGA
TTATGGCTTTACCTATATTGGTGCTTGGGTTTTTAGCCTTAAAAGGAGGACTTAGA
TTTACCATTTATTCTGTACCTGTAATGGCTTTAGGATTTGGTTTTTTATTGAGCGA
GTTTAAGGCTATATTGGTTAAAAAATATAGCCAATTAACTTCAAATGTTTGTATT
GTTTTTGCAACTATTTTGACTTTGGCTCCAGTATTTATCCATATTTACAACTATAA
AGCGCCAACAGTTTTTTCTCAAAATGAAGCATCATTATTAAATCAATTAAAAAAT
ATAGCCAATAGAGAAGATTATGTGGTAACTTGGTGGGATTATGGTTATCCTGTGC
GTTATTATAGCGATGTGAAAACTTTAGTAGATGGTGGAAAGCATTTAGGTAAGG
ATAATTTTTTCCCTTCTTTTTCTTTAAGTAAAGATGAACAAGCTGCAGCTAATATG
GCAAGACTTAGTGTAGAATATACAGAAAAAAGCTTTTATGCTCCGCAAAATGAT

ATTTTAAAATCAGACATTTTACAAGCCATGATGAAAGATTATAATCAAAGCAATG
TGGATTTATTTCTAGCTTCATTATCAAAACCTGATTTTAAAATCGATACACCAAA
AACTCGTGATATTTATCTTTATATGCCCGCTAGAATGTCTTTGATTTTTTCTACGG
TGGCTAGTTTTTCTTTTATTAATTTAGATACAGGAGTTTTGGATAAACCTTTTACC
TTTAGCACAGCTTATCCACTTGATGTTAAAAATGGAGAAATTTATCTTAGCAACG
GAGTGGTTTTAAGCGATGATTTTAGAAGTTTTAAAATAGGTGATAATGTGGTTTC
TGTAAATAGTATCGTAGAGATTAATTCTATTAAACAAGGTGAATACAAAATCACT
CCAATCGATGATAAGGCTCAGTTTTATATTTTTTATTTAAAGGATAGTGCTATTCC
TTACGCACAATTTATTTTAATGGATAAAACCATGTTTAATAGTGCTTATGTGCAA
ATGTTTTTTTTGGGAAATTATGATAAGAATTTATTTGACTTGGTGATTAATTCTAG
AGATGCTAAAGTTTTTAAACTTAAAATTTAA (SEQ ID NO: 57)

[00264] The amino acid sequence of the PglB protein encoded by the nucleic acid of SEQ ID NO: 57 is provided below:

MLKKEYLKNPYLVLFAMIVLAYVFSVFCRFYWVVWWASEFNEYFFNNQLMIISNDGY
AFAEGARDMIAGFHQPNDLSYYGSSLSTLTYWLYKITPFSFESIILYMSTFLSSLVVIPII
LLANEYKRPLMGFVAALLASVANSYYNRTMSGYYDTDMLVIVLPMFILFFMVRMIL
KKDFFSLIALPLFIGIYLWWYPSSYTLNVALIGLFLIYTLIFHRKEKIFYIAVILSSLTLSN
IAWFYQSAIIVILFALFALEQKRLNFMIIGILGSATLIFLILSGGVDPILYQLKFYIFRSDE
SANLTQGFMYFNVNQTIQEVENVDFSEFMRRISGSEIVFLFSLFGFVWLLRKHKSMIM
ALPILVLGFLALKGGLRFTIYSVPVMALGFGFLLSEFKAILVKKYSQLTSNVCIVFATI
LTLAPVFIHIYNYKAPTVFSQNEASLLNQLKNIANREDYVVTWWDYGYPVRYYSDV
KTLVDGGKHLGKDNFFPSFSLSKDEQAANMARLSVEYTEKSFYAPQNDILKSDILQA
MMKDYNQSNVDLFLASLSKPDFKIDTPKTRDIYLYMPARMSLIFSTVASFSFINLDTG
VLDKPFTFSTAYPLDVKNGEIYLSNGVVLSDDFRSFKIGDNVVSVNSIVEINSIKQGEY
KITPIDDKAQFYIFYLKDSAIPYAQFILMDKTMFNSAYVQMFFLGNYDKNLFDLVINS
RDAKVFKLKI (SEQ ID NO: 58)

[00265] In some embodiments, the nucleic acid comprises a *C. jejuni pglB* gene (provided as SEQ ID NO: 57). In some embodiments, the nucleic acid comprises a *pglB* gene, wherein the *pglB* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 57. In some embodiments, the nucleic acid

comprises a *pglB* gene, wherein the *pglB* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 57.

[00266] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglB protein, wherein said PglB protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 58. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglB protein, wherein said PglB protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 58.

[00267] *pglA* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglA* gene (*e.g.*, a *C. jejuni pglA* gene).

[00268] In some embodiments, the nucleic acid comprising a *pglA* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglA* gene is located on a chromosome of the bacterium.

[00269] The nucleic acid sequence of an exemplary *C. jejuni pglA* gene is provided below:

ATGAGAATAGGATTTTTATCACATGCAGGAGCGAGTATTTATCATTTTAGAATGC
CTATTATAAAAGCGTTAAAAGATAGAAAAGACGAAGTTTTTGTTATAGTGCCGCA
AGATGAATACACGCAAAAACTTAGAGATCTTGGCTTAAAAGTAATTGTTTATGAG
TTTTCAAGAGCTAGTTTAAATCCTTTTGTGGTTTTAAAGAATTTTTTTTATCTTGCT
AAGGTTTTGAAAAATTTAAATCTTGATTTTATTCAAAGTGCGGCACACAAAAGCA
ATACTTTTGGAATTTTAGCAGCAAAATGGGCAAAAATTCCTTATCGTTTTGCCTTA
GTAGAAGGCTTGGGATCTTTTTATATAGATCAAGGTTTTAAGGCAAATTTAGTGC
GTTTTGTTATTAATAGTCTTTATAAATTAAGTTTTAAATTTGCACACCAATTTATT
TTTGTCAATGAAAGTAATGCTGAGTTTATGCGGAATTTAGGACTTAAAGAAAATA

AAATTTGCGTGATAAAATCTGTAGGGATCAATTTAAAAAAATTTTTTCCTATTTAT
GTAGAATCGGAAAAAAAAGAGCTTTTTTGGAAAAATTTAAACATAGATAAAAAA
CCCATTGTGCTTATGATAGCAAGAGCTTTATGGCATAAGGGTGTAAAAGAATTTT
ATGAAAGTGCTACTATGCTAAAAGACAAAGCAAATTTTGTTTTAGTTGGTGGAAG
AGATGAAAATCCTTCTTGTGCAAGTTTGGAGTTTTTAAACTCTGGCGCGGTGCAT
TATTTGGGTGCTAGAAGTGATATAGTCGAGCTTTTGCAAAATTGTGATATTTTTGT
TTTGCCAAGCTATAAAGAAGGCTTTCCTGTAAGTGTTTTGGAGGCAAAAGCTTGC
GGTAAGGCTATAGTGGTGAGTGATTGTGAAGGTTGTGTGGAGGCTATTTCTAATG
CTTATGATGGACTTTGGGCAAAAACAAAAAATGCTAAAGATTTAAGCGAAAAAA
TTTCACTTTTATTAGAAGATGAAAAATTAAGATTAAATTTAGCTAAAAATGCCGC
CCAAGATGCTTTACAATACGATGAAAATATAATCGCACAGCGTTATTTAAAACTT
TATGATAGGGTAATTAAGAATGTA (SEQ ID NO: 59)

[00270] The amino acid sequence of the PglA protein encoded by the nucleic acid of SEQ ID NO: 59 is provided below:

MRIGFLSHAGASIYHFRMPIIKALKDRKDEVFVIVPQDEYTQKLRDLGLKVIVYEFSR
ASLNPFVVLKNFFYLAKVLKNLNLDFIQSAAHKSNTFGILAAKWAKIPYRFALVEGL
GSFYIDQGFKANLVRFVINSLYKLSFKFAHQFIFVNESNAEFMRNLGLKENKICVIKS
VGINLKKFFPIYVESEKKELFWKNLNIDKKPIVLMIARALWHKGVKEFYESATMLKD
KANFVLVGGRDENPSCASLEFLNSGAVHYLGARSDIVELLQNCDIFVLPSYKEGFPVS
VLEAKACGKAIVVSDCEGCVEAISNAYDGLWAKTKNAKDLSEKISLLLEDEKLRLNL
AKNAAQDALQYDENIIAQRYLKLYDRVIKNV (SEQ ID NO: 60)

[00271] In some embodiments, the nucleic acid comprises a *C. jejuni pglA* gene (provided as SEQ ID NO: 59). In some embodiments, the nucleic acid comprises a *pglA* gene, wherein the *pglA* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 59. In some embodiments, the nucleic acid comprises a *pglA* gene, wherein the *pglA* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 59.

[00272] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglA protein, wherein said PglA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 60. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglA protein, wherein said PglA protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 60.

[00273] *pglC* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglC* gene (*e.g.*, a *C. jejuni pglC* gene).

[00274] In some embodiments, the nucleic acid comprising a *pglC* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglC* gene is located on a chromosome of the bacterium.

[00275] The nucleic acid sequence of an exemplary *C. jejuni pglC* gene is provided below:

GAAAAAGTTTTTAAAAGAATTTTTGATTTTATTTTATCTTTAGTGCTTTTAGTGCT
TTTTTCTCCGGTGATTTTAATCACTGCTTTACTTTTAAAAATCACTCAAGGAAGTG
TGATTTTTACCCAAAATCGTCCCGGGTTAGATGAAAAAATTTTTAAAATTTATAA
ATTTAAAACCATGAGCGATGAAAGAGATGAAAAGGGTGAGTTATTAAGCGATGA
ATTGCGTTTGAAAGCTTTTGGAAAAATCGTTAGAAGCTTAAGTTTGGATGAGCTT
TTGCAACTTTTTAATGTTTTAAAAGGGGATATGAGTTTTGTTGGACCTAGACCTCT
TTTGGTTGAGTATTTGCCTCTTTACAATAAAGAGCAAAAATTGCGTCATAAAGTG
CGTCCAGGTATAACAGGATGGGCGCAGGTAAATGGTAGAAATGCTATTTCTTGG
CAGAAAAAATTCGAACTTGATGTGTATTATGTGAAAAATATTTCTTTTTTGCTTGA
TTTAAAAATCATGTTTTTAACAGCTTTAAAGGTTTTAAAACGAAGTGGGGTAAGC
AAAGAAGGCCATGTTACAACAGAGAAATTTAATGGCAAGAACTGA (SEQ ID NO: 61)

[00276] The amino acid sequence of the PglC protein encoded by the nucleic acid of SEQ ID NO: 61 is provided below:
EKVFKRIFDFILSLVLLVLFSPVILITALLLKITQGSVIFTQNRPGLDEKIFKIYKFKTMS
DERDEKGELLSDELRLKAFGKIVRSLSLDELLQLFNVLKGDMSFVGPRPLLVEYLPLY
NKEQKLRHKVRPGITGWAQVNGRNAISWQKKFELDVYYVKNISFLLDLKIMFLTAL
KVLKRSGVSKEGHVTTEKFNGKN (SEQ ID NO: 62)

[00277] In some embodiments, the nucleic acid comprises a *C. jejuni pglC* gene (provided as SEQ ID NO: 61). In some embodiments, the nucleic acid comprises a *pglC* gene, wherein the *pglC* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO:61. In some embodiments, the nucleic acid comprises a *pglC* gene, wherein the *pglC* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 61.

[00278] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglC protein, wherein said PglC protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 62. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglC protein, wherein said PglC protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 62.

[00279] *pglD* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglD* gene (*e.g.*, a *C. jejuni pglD* gene).

[00280] In some embodiments, the nucleic acid comprising a *pglD* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglD* gene is located on a chromosome of the bacterium.

[00281] The nucleic acid sequence of an exemplary *C. jejuni pglD* gene is provided below:

ATGGCAAGAACTGAAAAAATTTATATTTATGGTGCTAGTGGTCATGGGCTTGTTTGTGAAGA
TGTGGCTAAAAATATGGGTTATAAAGAATGTATTTTTTTAGATGATTTTAAAGGAATGAAAT
TTGAAAGTACCTTACCTAAATATGATTTTTTTATAGCCATAGGAAACAATGAAATTCGAAAA
AAGATTTATCAAAAAATTTCAGAAAATGGCTTTAAAATTGTCAATCTTATCCATAAAAGCGC
GCTTATAAGTCCTAGCGCAATCGTGGAAGAAAATGCAGGAATTTTAATCATGCCTTATGTAG
TGATTAACGCTAAAGCTAAAATAGAAAAAGGTGTGATTTTAAATACTTCAAGCGTAATTGAG
CATGAATGTGTGATAGGGGAATTTTCTCATGTGAGTGTGGGAGCTAAATGTGCGGGTAATGT
AAAAATTGGTAAAAATTGTTTTTTAGGGATTAATTCTTGTGTTTTGCCTAATTTAAGTTTGG
CAGATGATAGTATTTTAGGTGGTGGAGCAACTTTAGTTAAAAATCAAGATGAAAAAGGTGTT
TTTGTGGGAGTACCTGCAAAAAGGATGTAA (SEQ ID NO: 63)

[00282] The amino acid sequence of the PglD protein encoded by the nucleic acid of SEQ ID NO: 63 is provided below:

MARTEKIYIYGASGHGLVCEDVAKNMGYKECIFLDDFKGMKFESTLPKYDFFIAIGNNEIRK
KIYQKISENGFKIVNLIHKSALISPSAIVEENAGILIMPYVVINAKAKIEKGVILNTSSVIE
HECVIGEFSHVSVGAKCAGNVKIGKNCFLGINSCVLPNLSLADDSILGGGATLVKNQDEKGV
FVGVPAKRM (SEQ ID NO: 64)

[00283] In some embodiments, the nucleic acid comprises a *C. jejuni pglD* gene (provided as SEQ ID NO: 63). In some embodiments, the nucleic acid comprises a *pglD* gene, wherein the *pglD* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 63. In some embodiments, the nucleic acid comprises a *pglD* gene, wherein the *pglD* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 63.

[00284] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglD protein, wherein said PglD protein comprises an amino acid

sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 64. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglD protein, wherein said PglD protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 64.

[00285] *pglE* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglE* gene (*e.g.*, a *C. jejuni pglE* gene).

[00286] In some embodiments, the nucleic acid comprising a *pglE* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglE* gene is located on a chromosome of the bacterium.

[00287] The nucleic acid sequence of an exemplary *C. jejuni pglE* gene is provided below:

ATGAGATTTTTTCTTTCTCCTCCGCATATGGGTGGTAATGAATTAAAATACATAG
AAGAAGTTTTCAAAAGCAATTATATAGCACCTTTGGGTGAATTTGTAAATCGCTT
TGAACAAAGTGTCAAGGCTTACAGTAAAAGTGAAAATGCCTTAGCTTTAAATTCA
GCCACAGCGGCTTTGCATTTAGCTTTAAGGGTGGCAGGGGTAAAACAAGATGAT
ATTGTTTTGGCTTCTTCTTTTACTTTTATCGCTTCAGTAGCACCTATTTGTTATCTT
AAAGCAAAACCTGTATTTATAGATTGTGATGAAACTTATAATATCGATGTAGATT
TATTAAAGCTTGCTATTAAAGAATGTGAAAAAAAACCAAAAGCATTGATTTTAAC
TCATCTTTATGGCAATGCGGCTAAAATGGATGAAATTGTTGAAATTTGCAAAGAA
AATGAAATTGTTTTAATCGAAGATGCTGCTGAAGCTTTAGGAAGTTTTTATAAGA
ATAAAGCTTTAGGAACTTTTGGAGAATTTGGAGCTTATTCTTATAATGGCAATAA
AATTATCACCACTTCAGGTGGAGGTATGCTTATAGGAAAAAATAAAGAAAAGAT
TGAAAAAGCAAGATTTTATAGCACTCAAGCTAGGGAAAATTGTTTGCATTATGAA
CATTTAGATTATGGTTATAATTACCGCTTAAGCAATGTTTTAGGAGCTATTGGCGT
AGCGCAAATGGAGGTTTTAGAACAAAGAGTGCTTAAAAAAAGAGAAATTTATGA
GTGGTATAAAGAATTTTTAGGAGAGTGTTTTAGCTTTTTAGATGAATTAGAAAAT
TCAAGAAGCAATCGCTGGTTAAGTACAGCTTTGATTGATTTTGATAAAAATGAAC

TTAATTCTTGTCAAAAAGATATAAATATCAGTCAAAAAAATATTACTTTGCATCC
AAAAATTTCAAAACTCATAGAAGATTTGAAAAATGAACAAATAGAAACAAGACC
ATTATGGAAAGCTATGCACGCTCAAGAAGTATTTAAAGGAGCTAAGGCTTATCTT
AATGGCAATAGTGAGTTATTTTTCCAAAAAGGAATTTGTTTGCCAAGTGGCACGG
CGATGAGTAAAGATGATGTTTATGAAATTTCAAAACTGATCTTAAAGAGCATAA
AGGCTTAA (SEQ ID NO: 65)

[00288] The amino acid sequence of the PglE protein encoded by the nucleic acid of SEQ ID NO: 65 is provided below:

MRFFLSPPHMGGNELKYIEEVFKSNYIAPLGEFVNRFEQSVKAYSKSENALALNSAT
AALHLALRVAGVKQDDIVLASSFTFIASVAPICYLKAKPVFIDCDETYNIDVDLLKLAI
KECEKKPKALILTHLYGNAAKMDEIVEICKENEIVLIEDAAEALGSFYKNKALGTFGE
FGAYSYNGNKIITTSGGGMLIGKNKEKIEKARFYSTQARENCLHYEHLDYGYNYRLS
NVLGAIGVAQMEVLEQRVLKKREIYEWYKEFLGECFSFLDELENSRSNRWLSTALID
FDKNELNSCQKDINISQKNITLHPKISKLIEDLKNEQIETRPLWKAMHAQEVFKGAKA
YLNGNSELFFQKGICLPSGTAMSKDDVYEISKLILKSIKA (SEQ ID NO: 66)

[00289] In some embodiments, the nucleic acid comprises a *C. jejuni pglE* gene (provided as SEQ ID NO: 65). In some embodiments, the nucleic acid comprises a *pglE* gene, wherein the *pglE* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 65. In some embodiments, the nucleic acid comprises a *pglE* gene, wherein the *pglE* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 65.

[00290] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglE protein, wherein said PglE protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 66. In some

embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglE protein, wherein said PglE protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 66.

[00291] *pglF* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglF* gene (*e.g.*, a *C. jejuni pglF* gene).

[00292] In some embodiments, the nucleic acid comprising a *pglF* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *pglF* gene is located on a chromosome of the bacterium.

[00293] The nucleic acid sequence of an exemplary *C. jejuni pglF* gene is provided below:

ATGATTTTTTATAAAAGCAAAAGATTAGCATTTTTTTTAACTTCAGATATTGTTTT
AATTTTACTTAGCGTTTATCTGGCTTTTTCTTTGAGATTTAGTGGAGATATTCCGA
GTATTTTTTATCATGGTATGATGGTTTCTGCTATTATTTTGCTTGTTTTAAAACTTT
CATTTTTGTTTGTTTTTAGAATTTATAAAGTAGCTTGGAGATTTTTTTCTCTCAATG
AAGCAAGAAAGATTTTTATCGCTTTGCTTTTAGCTGAGTTTTGTTTTTTTCTTATTT
TTTATTTTTTTAGTGATTTTTTTAATCCTTTTCCAAGAAGTGCTATTGTGATAGATT
TTGTTCTTTCTTATATGTTTATAGGTACTTTAAGAATTAGCAAAAGAATGCTTGTG
GATTTTAAACCTTCTAGAATGAAAGAAGAAGAAACTCCTTGTATTGTAGTAGGGG
CAACTTCTAAGGCTTTGCATTTGTTAAAAGGTGCAAAAGAAGGTTCTTTAGGGCT
TTTTCCTGTAGGCGTAGTTGATGCGAGAAAAGAGCTTATAGGGACTTATTGTGAT
AAATTTATTGTAGAAGAAAAAGAAAAAATAAAATCTTATGTAGAACAAGGGGTA
AAAACTGCCATTATTGCTTTAAGACTTGAACAAGAAGAGCTTAAAAAACTTTTTG
AAGAACTTGTAGCTTATGGTATTTGCGATGTAAAAATATTTTCTTTTACAAGAAA
CGAAGCAAGAGATATCAGTATAGAAGACTTGCTTGCTAGAAAACCAAAAGATTT
AGATGATAGTGCTGTGGCGGCTTTTTTAAAAGATAAGGTAGTTTTGGTAAGTGGA
GCAGGTGGAACTATAGGCAGTGAACTTTGTAAGCAATGTATTAAATTTGGTGCTA
AGCATCTTATCATGGTTGATCATAGTGAGTATAATCTTTATAAGATCAATGATGA
TTTAAATTTATATAAAGAAAAAATTACTCCTATTTTACTGAGTATTTTAGATAAGC
AAAGTTTAGATGAGGTATTAAAAACTTATAAACCCGAGCTTATTTTACATGCAGC
CGCTTATAAACATGTGCCTCTTTGCGAACAAAATCCACATTCAGCAGTAATCAAT

AATATTTTAGGAACTAAAATTTTATGCGACAGTGCTAAAGAAAACAAAGTAGCT
AAATTTGTGATGATAAGTACAGATAAAGCAGTACGACCAACAAATATTATGGGT
TGCACTAAGAGAGTTTGCGAGCTTTATACTTTAAGTATGAGTGATGAAAATTTTG
AAGTTGCTTGTGTGCGTTTTGGTAATGTTTTAGGTTCTAGTGGTAGTGTGATACCG
AAATTTAAAGCACAAATTGCCAATAATGAGCCTTTAACTTTAACGCACCCTGATA
TAGTGCGTTATTTTATGCTTGTGGCTGAGGCAGTGCAACTTGTTTTACAAGCTGG
AGCTATCGCAAAAGGGGGAGAACTTTTTGTTTTGGATATGGGTAAGCCTGTGAAA
ATCATAGATTTAGCTAAAAAAATGCTTTTACTTTCTAATCGCAATGATTTAGAAA
TTAAAATCACAGGCTTAAGAAAAGGTGAGAAGCTTTATGAAGAGCTTTTGATTG
ATGAAAATGATGCTAAAACACAATATGAGAGTATTTTTGTAGCAAAGAATGAGA
AGGTTGATCTTGATTGGCTTAATAAAGAGATAGAAAATTTACAAATATGTGAAG
ATATTTCAGAGGCTTTATTAAAGATTGTACCTGAATTTAAACACAATAAAGAAGG
TGTA (SEQ ID NO: 67)

[00294] The amino acid sequence of the PglF protein encoded by the nucleic acid of SEQ ID NO: 67 is provided below:

MIFYKSKRLAFFLTSDIVLILLSVYLAFSLRFSGDIPSIFYHGMMVSAIILLVLKLSFLFV
FRIYKVAWRFFSLNEARKIFIALLLAEFCFFLIFYFFSDFFNPFPRSAIVIDFVLSYMFIG
TLRISKRMLVDFKPSRMKEEETPCIVVGATSKALHLLKGAKEGSLGLFPVGVVDARK
ELIGTYCDKFIVEEKEKIKSYVEQGVKTAIIALRLEQEELKKLFEELVAYGICDVKIFSF
TRNEARDISIEDLLARKPKDLDDSAVAAFLKDKVVLVSGAGGTIGSELCKQCIKFGA
KHLIMVDHSEYNLYKINDDLNLYKEKITPILLSILDKQSLDEVLKTYKPELILHAAAY
KHVPLCEQNPHSAVINNILGTKILCDSAKENKVAKFVMISTDKAVRPTNIMGCTKRV
CELYTLSMSDENFEVACVRFGNVLGSSGSVIPKFKAQIANNEPLTLTHPDIVRYFMLV
AEAVQLVLQAGAIAKGGELFVLDMGKPVKIIDLAKKMLLLSNRNDLEIKITGLRKGE
KLYEELLIDENDAKTQYESIFVAKNEKVDLDWLNKEIENLQICEDISEALLKIVPEFKH
NKEGV (SEQ ID NO: 68).

[00295] In some embodiments, the nucleic acid comprises a *C. jejuni pglF* gene (provided as SEQ ID NO: 67). In some embodiments, the nucleic acid comprises a *pglF* gene, wherein the *pglF* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 67. In some embodiments, the nucleic acid

comprises a *pglF* gene, wherein the *pglF* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 67.

[00296] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglF protein, wherein said PglF protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:68. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglF protein, wherein said PglF protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 68.

[00297] *pglG* - In some embodiments, the recombinant bacterium comprises a nucleic acid comprising a *pglG* gene (*e.g.*, a *C. jejuni pglG* gene).

[00298] In some embodiments, the nucleic acid comprising a *pglG* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising an *pglG* gene is located on a chromosome of the bacterium.

[00299] The nucleic acid sequence of an exemplary *C. jejuni pglG* gene is provided below:

ATGTATATAAAAGATATACAAAGATTTGAAGATAATCGCTATCGTGCTAGAGCTT
ATATGAGTTATATTTTAACAAGAAATCTGCCCAATAAACTTCCTGATATTCACCTT
GAAACGATTAAAACAGCTTTGGATAAAATAGCTCATGAAGTTGTTGTTTTTGATG
CTTTGTATATTTTAGATATTTCAGGCATGCAAATAGAAAATGCGATTTCCTTAAAT
AAAGCTCATGAAATAGGGCAGGGTGAGGATAGAAGTACTCGTTCTTATTTTTATA
GAGCTGTAAAATTAAGACGATGTGTTTTGAGCGATCCTTATCCTTCGGTTTTAAA
TAATGAGCTTTGCGTGACAGCTTCTATGCCAATTTACGATGATAAAAATAACTTG
CTTTTTGTTGTTTGTATTGATATCAAGCTTGAAGATATTTTAAAGATTATTCAAGC
AGGAAAATTTGAATTTGTTTTTACTCAGTTTAGTCGTTTGGTATATTTTTGCTTCG

CACTGGTTTTATTTGTGATTACTTGTTTTTTATTTCAAAAAGGTTTTTTTAGTCTTT
TTGATAATCAAGCTATAGGTATAGAACATATGTTTGAAAGTACCATCGCTATAAC
TTTGGCTTTAGCTATTTTTGATTTGGCAAAAACTTTGATCGAACAAGAAGTATTA
GGAAGGACGAAAAAAGAAGAAGGTGGAATTCAAAAAACTATGGTGAGATTTTTG
GGTTCTATTATCATTGCTTTAGCTATAGAAGCTTTGATGTTGGTATTTAAACTTGC
TATTGGTGATCTTTCTCAGATGATTTATGCGATTTATCTTATCGGTGGAGTGAGCT
TGCTTCTTTTAGGTTTAAGTGTATATTTATTTACGGTTAAGTATAAAAATAATAAT
ATTTGA (SEQ ID NO: 69)

[00300] The amino acid sequence of the PglG protein encoded by the nucleic acid of SEQ ID NO: 69 is provided below:

MYIKDIQRFEDNRYRARAYMSYILTRNLPNKLPDIHLETIKTALDKIAHEVVVFDALY
ILDISGMQIENAISLNKAHEIGQGEDRSTRSYFYRAVKLRRCVLSDPYPSVLNNELCV
TASMPIYDDKNNLLFVVCIDIKLEDILKIIQAGKFEFVFTQFSRLVYFCFALVLFVITCF
LFQKGFFSLFDNQAIGIEHMFESTIAITLALAIFDLAKTLIEQEVLGRTKKEEGGIQKTM
VRFLGSIIIALAIEALMLVFKLAIGDLSQMIYAIYLIGGVSLLLLGLSVYLFTVKYKNN
NI (SEQ ID NO: 70)

[00301] In some embodiments, the nucleic acid comprises a *C. jejuni pglG* gene (provided as SEQ ID NO: 69). In some embodiments, the nucleic acid comprises a *pglG* gene, wherein the *pglG* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 69. In some embodiments, the nucleic acid comprises a *pglG* gene, wherein the *pglG* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 69.

[00302] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglG protein, wherein said PglG protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 70. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a PglG protein, wherein said PglG protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 70.

C. Attenuation

[00303] In some embodiments, the recombinant bacterium described herein is modified such that the expression of one or more genes can be regulated in a sugar-responsive manner. In some embodiments, one or more endogenous genes, *e.g.,* virulence genes, are deleted from the bacterial chromosome. In some embodiments, the deletion is a partial deletion of the endogenous gene. In some embodiments, the deletion is a full-length deletion of the endogenous gene. In some embodiments, the gene is genetically-altered to prevent transcription and/or translation of the gene encoding the protein. In some embodiments, the endogenous gene is genetically altered to insert a transcriptional terminator in the open reading frame of the gene. In some embodiments, a regulatory region of the gene, *e.g.,* virulence gene, is genetically-modified to alter (*e.g.*, decrease) the expression of the gene. In some embodiments, the promoter of a gene, *e.g.,* virulence gene, is altered to include one or more regulatory elements (*e.g.*, a sugar-responsive promoter).

[00304] In some embodiments, the recombinant bacterium described herein is modified to comprise a nucleic acid comprising a gene. In some embodiments, the recombinant bacterium is modified to comprise a nucleic acid comprising a gene, whereby an endogenous copy of the gene in the bacterial chromosome has been altered and/or deleted. In some embodiments, the nucleic acid comprises a gene that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an endogenous gene in the bacterial chromosome that has been deleted and/or altered. In some embodiments, the nucleic acid comprises a gene that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to an endogenous gene in the bacterial chromosome that has been deleted and/or altered. In some embodiments, the nucleic acid comprises a gene from a bacterial species, subspecies, serovar, or strain that is different than the bacterial species of the recombinant bacterium.

[00305] In some embodiments, the nucleic acid comprises a gene from a bacterial species, subspecies, serovar, or strain that is the same as the bacterial species of the recombinant bacterium. In some embodiments, the nucleic acid comprises a gene that is operably-linked to a regulatable promoter (*e.g.*, a sugar-regulatable promoter). In some embodiments, the nucleic acid comprises a gene that is operably-linked to a rhamnose-regulatable promoter, a xylose-regulatable promoter, a galactose-regulatable promoter, an arabinose-regulatable promoter, a rhamnose-regulatable promoter, a mannose-regulatable promoter, or a maltose-regulatable promoter. In some embodiments, the nucleic acid comprising the gene is located in a plasmid in the bacterium. In some embodiments, the nucleic acid comprising the gene is located in the bacterial chromosome. In some embodiments, the nucleic acid comprising the gene is located at the chromosomal locus corresponding to the locus of an endogenous gene that has been deleted or altered in the bacterial chromosome. In some embodiments, the nucleic acid is codon-optimized (*e.g.,* to improve expression of the nucleic acid in the recombinant bacterium).

GTP pyrophosphokinase genes

[00306] In some embodiments, the recombinant bacterium comprises a deletion in an endogenous *relA* gene, which encodes the GTP pyrophosphokinase RelA. The inclusion of a relA deletion in the recombinant bacterium uncouples the occurrence of growth-dependent lysis to the need for continued protein synthesis. In some embodiments, the deletion of the endogenous *relA* gene is a partial deletion. In some embodiments, the deletion of the endogenous *relA* gene is a full-length deletion.

Other attenuation methods

[00307] Other methods of attenuation are known in the art. For instance, attenuation may be accomplished by altering (*e.g.*, deleting) native nucleic acid sequences found in the wild type bacterium. For instance, if the bacterium is *Salmonella*, non-limiting examples of nucleic acid sequences which may be used for attenuation include: a *pab* nucleic acid sequence, a *pur* nucleic acid sequence, an *aro* nucleic acid sequence, *asd*, a *dap* nucleic acid sequence, *nadA*, *pncB*, *galE*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *galU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hilA*, *rpoE*, *flgM*, *tonB*, *slyA*, and

any combination thereof. Exemplary attenuating mutations may be *aroA*, *aroC*, *aroD*, *cdt*, *cya*, *crp*, *phoP*, *phoQ*, *ompR*, *galE*, and *htrA*.

[00308] In certain embodiments, the above nucleic acid sequences may be placed under the control of a sugar regulated promoter wherein the sugar is present during *in vitro* growth of the recombinant bacterium, but substantially absent within an animal or human host. The cessation in transcription of the nucleic acid sequences listed above would then result in attenuation and the inability of the recombinant bacterium to induce disease symptoms.

D. Additional Mutations

[00309] In some embodiments, the recombinant bacterium comprises a deletion in an endogenous *recF* gene, which encodes the DNA replication and repair protein RecF. In some embodiments, the deletion of the endogenous *recF* gene is a partial deletion. In some embodiments, the deletion of the endogenous *recF* gene is a full-length deletion. In some embodiments, the endogenous *recF* gene is genetically altered to insert a transcriptional terminator in the open reading frame of the gene.

[00310] In some embodiments, the recombinant bacterium comprises a deletion in an endogenous *recJ* gene, which encodes the exonuclease RecJ. In some embodiments, the deletion of the endogenous *recJ* gene is a partial deletion. In some embodiments, the deletion of the endogenous *recJ* gene is a full-length deletion. In some embodiments, the endogenous *recJ* gene is genetically altered to insert a transcriptional terminator in the open reading frame of the gene.

[00311] The bacterium may also be modified to create a balanced-lethal host-vector system, although other types of systems may also be used (*e.g.*, creating complementation heterozygotes). For the balanced-lethal host-vector system, the bacterium may be modified by manipulating its ability to synthesize various essential constituents needed for synthesis of the rigid peptidoglycan layer of its cell wall. In one example, the constituent is diaminopimelic acid (DAP) (see, for example, U.S. Patent Nos. 5,672,345; 5,840,482; and 6,872,547, the entire contents of each of which are expressly incorporated herein by reference). Various enzymes are involved in the eventual synthesis of DAP.

[00312] In some embodiments, the recombinant bacterium comprises a deletion in an endogenous *asd* gene. In some embodiments, the deletion of the endogenous *asd* gene is a partial deletion. In some embodiments, the deletion of the endogenous *asd* gene is a full-

length deletion. In some embodiments, the endogenous *asd* gene is genetically altered to insert a transcriptional terminator in the open reading frame of the gene. In some embodiments, the promoter of an endogenous *asd* gene is altered to include one or more regulatory elements (*e.g.*, a sugar-responsive promoter). In one example, the bacterium is modified by using a Δ*asdA* mutation to eliminate the bacterium's ability to produce β-aspartate semialdehyde dehydrogenase, an enzyme essential for the synthesis of DAP. Other mutations that result in the abolition of the synthesis of DAP include, but are not limited to, *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dapF*, and *asd* (see, *e.g.*, U.S. Patent No. 6,872,547, incorporated herein by reference). Other modifications that may be employed include modifications to a bacterium's ability to synthesize D-alanine or to synthesize D-glutamic acid (*e.g.*, Δ*murI* mutations), which are both unique constituents of the peptidoglycan layer of the bacterial cell wall.

[00313] Similarly, various embodiments may comprise the *araC* P_{araBAD} *c2* gene cassette inserted into the *asd* nucleic acid sequence that encodes aspartate semialdehyde dehydrogenase. Since the *araC* nucleic acid sequence is transcribed in a direction that could lead to interference in the expression of adjacent nucleic acid sequences and adversely affect vaccine strain performance, a transcription termination (TT) sequence is generally inserted 3' to the *araC* nucleic acid sequence. The chromosomal *asd* nucleic acid sequence is typically inactivated to enable use of plasmid vectors encoding the wild-type *asd* nucleic acid sequence in the balanced lethal host-vector system. This allows for stable maintenance of plasmids *in vivo* in the absence of any drug resistance attributes that are not permissible in live bacterial vaccines. In some of these embodiments, the wild-type *asd* nucleic acid sequence may be encoded by the vector described herein. The vector enables the regulated expression of an antigen encoding sequence through the repressible promoter.

Regulated Delayed Lysis in vivo

[00314] In some embodiments, the recombinant bacterium may comprise deletions and/or deletion-insertion mutations to facilitate regulated delayed lysis *in vivo* that prevents bacterial persistence *in vivo* and survival if excreted (**Table 3**). These chromosomal mutations may include: Δ(*wza-wcaM*), ΔP_{murA}::TT *araC* P_{BAD} *murA,* and Δ*asdA*::TT *araC* P_{BAD} *c2.* Δ(*wza-wcaM*) eliminates twenty enzymes needed to synthesize several exopolysaccharides that promote biofilm formation and for synthesis of GDP-fucose that is required for colonic acid synthesis 9194), which can protect cells from undergoing cell wall-less death from lysing (195). ΔP_{murA}::TT *araC* P_{BAD} *murA*, makes synthesis of MurA, the

first enzyme in the synthesis of muramic acid, dependent on presence of arabinose in growth medium and ceases to be synthesized *in vivo* due to the absence of arabinose (142) (118). MurA decreases as a consequence of cell division *in vivo* to ultimately lead to cell lysis and death (166) (191). The *murA* defect is complemented by $MurA^+$ plasmid vectors (142) (118). With respect to the Δ*asdA*::TT *araC* P_{BAD} *c2* mutations, the Asd enzyme is essential for the synthesis of diaminopimelic acid required for peptidoglycan synthesis (167) (192). The arabinose-dependent synthesis of the C2 repressor is to enable a regulated delayed expression of DNA sequences under the control of a promoter repressed by C2 (142) (118). The Δ*asdA* mutation is complemented by Asd^+ plasmid vectors (157) (193).

[00315] The latter two mutations are typically complemented by a regulated delayed lysis plasmid vector that has an arabinose-dependent expression of *asdA* and *murA* genes. A recombinant bacterium comprising such mutations grows normally in the presence of arabinose. In vivo, however, the bacterium ceases to express any nucleic acids encoding the Asd and MurA enzymes, such that synthesis of the peptidoglycan cell wall layer ceases, ultimately resulting in the lysis of the bacterium. This lysis may result in the release of a bolus of antigen specific for an enteric pathogen, thereby serving as a means to enhance induction of immunity against that enteric pathogen while conferring biological containment.

[00316] Additional methods of delayed lysis are described in, for example, U.S. Patent No. 9,481,888, the entire contents of which are expressly incorporated herein by reference.

sopB mutation

[00317] To be safe for use as a vaccine, the bacterial enteric pathogen must be attenuated for virulence by deletion or regulated expression of a virulence gene. In the case of *Salmonella*, for instance, an effector molecule secreted by the type 3 secretion system, such as *sopB*, may be altered to achieve attenuation. The genes may be deleted or a regulatable promoter may be inserted in front of the gene to achieve regulated delayed attenuation. As used herein, "regulated delayed attenuation" refers to the ability of the microbe to colonize a host and then display an attenuation phenotype to avoid actually causing a symptomatic infection.

E. Repressor Regulatory Systems

[00318] In some embodiments, the recombinant bacterium comprises a nucleic acid (*e.g.*, a gene) that is operably linked to a repressor-regulatable promoter to facilitate the

regulatable expression of the gene. Thus, in some embodiments, the recombinant bacterium comprises a nucleic acid comprising a gene encoding a repressor. In some embodiments, the gene encoding the repressor is operably-linked to a regulatable promoter. Methods of chromosomally integrating a nucleic acid sequence encoding a repressor operably-linked to a regulatable promoter are known in the art and detailed in the examples. In some embodiments, the nucleic acid sequence encoding a repressor is not integrated into a chromosomal locus such that the ability of the bacterium to colonize a host cell is disrupted. In some embodiments, the recombinant bacterium comprises a nucleic acid encoding a repressor that is integrated into the *relA* locus of the bacterial chromosome. In some embodiments, the recombinant bacterium comprises a nucleic acid encoding a repressor that is integrated into the *endA* locus of the bacterial chromosome. In some embodiments, the recombinant bacterium comprises at least one nucleic acid sequence encoding a repressor. In some embodiments, the recombinant bacterium comprises at least two, at least three, at least four, at least five, at least six or more nucleic acids encoding a repressor. In some embodiments, the nucleic acid encoding the repressor is present on a plasmid in the bacterium. In some embodiments, the nucleic acid encoding the repressor is located in the bacterial chromosome. If there is more than one nucleic acid sequence encoding a repressor, each nucleic acid sequence encoding a repressor may be operably linked to a regulatable promoter, such that each promoter is regulated by the same compound or condition. Alternatively, each nucleic acid sequence encoding a repressor may be operably linked to a regulatable promoter, each of which is regulated by a different compound or condition.

[00319] As used herein, a "repressor" refers to a biomolecule that represses the transcriptional activity of a promoter. In some embodiments, the repressor is synthesized by the recombinant bacterium in high enough quantities during *in vitro* culture, such that the transcription of a nucleic acid that is operably linked to a repressor-regulatable promoter is repressed. This may be particularly advantageous if, for example, expression of the product encoded by said nucleic acid impedes the *in vitro* growth of the bacterium, and/or the ability of the bacterium to infect and/or colonize a subject. In some embodiments, the nucleic acid that is operably-linked to the repressor-regulatable promoter expresses an antigen of interest. In some embodiments, the concentration of the repressor within the cell gradually decreases with each cell division cycle after transcription of the gene encoding the repressor decreases or ceases (*e.g.*, *in vivo*). The use of a particular repressor, as described herein, may depend, in part, on the species, subspecies, strain or serovar of the recombinant bacterium being used.

In some embodiments, the repressor is derived from the same species (*e.g.*, the same bacterial species or the same phage) from which the repressor-regulatable promoter is derived. In some embodiments the repressor is not derived from the same bacterial species as the bacterial species in which the repressor is expressed. For example, in some embodiments, the repressor is derived from *E. coli* if the recombinant bacterium is of the genus *Salmonella*. Other suitable repressors include repressors derived from a bacteriophage.

[00320] A nucleic acid sequence encoding a repressor and regulatable promoter detailed above may be modified so as to optimize the expression level of the nucleic acid sequence encoding the repressor. The optimal level of expression of the nucleic acid sequence encoding the repressor may be estimated, or may be determined by experimentation. Such a determination should take into consideration whether the repressor acts as a monomer, dimer, trimer, tetramer, or higher multiple, and should also take into consideration the copy number of the vector encoding the antigen of interest. In an exemplary embodiment, the level of expression is optimized so that the repressor is synthesized while in a permissive environment (*i.e.*, *in vitro* growth) at a level that substantially inhibits the expression of the nucleic acid encoding an antigen of interest, and is substantially not synthesized in a non-permissive environment, thereby allowing expression of the nucleic acid encoding an antigen of interest.

[00321] In some embodiments, the recombinant bacterium described herein is modified to comprise a nucleic acid comprising a *lacI* gene, which encodes the LacI repressor protein. The expression of the *lacI*-encoded repressor in the recombinant bacterium described herein may be used to regulate the expression of an antigen of interest expressed by the bacterium. For example, in some embodiments, the expression of the *lacI* gene is regulated by a sugar-regulatable promoter (*e.g.*, an arabinose-regulatable promoter). When cultured in the presence of arabinose, the recombinant bacterium will express the LacI repressor protein, which in turn will repress the expression of a gene encoding an antigen of interest that is operably-linked to a LacI-responsive promoter (*e.g.*, P_{trc}, P_{lac}, P_{T7lac} and P_{tac}). Upon administration to the subject and in the absence of a source of arabinose, the synthesis of LacI repressor ceases, leading to de-repression of the LacI-responsive promoter and the subsequence expression of the antigen of interest. The concentration of LacI in the cell decreases by about half at each cell division *in vivo*, leading to a gradual decreased level of repression and gradual increased synthesis of the antigen of interest.

[00322] In some embodiments, the nucleic acid comprising a *lacI* gene is located on a plasmid in the bacterium. In some embodiments, the nucleic acid comprising a *lacI* gene is located on a chromosome of the bacterium. In some embodiments, the nucleic acid comprising a *lacI* gene is located at the chromosomal locus corresponding to the locus of an endogenous a *relA* gene that has been deleted or altered in the bacterial chromosome. In some embodiments, the recombinant bacterium is modified to comprise a nucleic acid comprising a *lacI* gene, whereby an endogenous copy of the *lacI* gene in the bacterial chromosome has been altered and/or deleted.

[00323] In some embodiments, the nucleic acid comprises a *Escherichia coli lacI* gene. The nucleic acid sequence of the *E. coli lacI* gene is provided below:
gtgaaaccagtaacgttatacgatgtcgcagagtatgccggtgtctcttatcagaccgtttcccgcgtggtgaaccaggccagccacgt
ttctgcgaaaacgcgggaaaaagtggaagcggcgatggcggagctgaattacattcccaaccgcgtggcacaacaactggcgggc
aaacagtcgttgctgattggcgttgccacctccagtctggccctgcacgcgccgtcgcaaattgtcgcggcgattaaatctcgcgccga
tcaactgggtgccagcgtggtggtgtcgatggtagaacgaagcggcgtcgaagcctgtaaagcggcggtgcacaatcttctcgcgca
acgcgtcagtgggctgatcattaactatccgctggatgaccaggatgccattgctgtggaagctgcctgcactaatgttccggcgttattt
cttgatgtctctgaccagacacccatcaacagtattattttctcccatgaagacggtacgcgactgggcgtggagcatctggtcgcattg
ggtcaccagcaaatcgcgctgttagcgggcccattaagttctgtctcggcgcgtctgcgtctggctggctggcataaatatctcactcgc
aatcaaattcagccgatagcggaacgggaaggcgactggagtgccatgtccggttttcaacaaaccatgcaaatgctgaatgagggc
atcgttcccactgcgatgctggttgccaacgatcagatggcgctgggcgcaatgcgcgccattaccgagtccgggctgcgcgttggtg
cggatatctcggtagtgggatacgacgataccgaagacagctcatgttatatcccgccgttaaccaccatcaaacaggattttcgcctgc
tggggcaaaccagcgtggaccgcttgctgcaactctctcagggccaggcggtgaagggcaatcagctgttgcccgtctcactggtga
aaagaaaaaccaccctggcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggttt
cccgactggaaagcgggcagtga (SEQ ID NO: 78)

[00324] The amino acid sequence of the *E. coli* LacI protein encoded by the nucleic acid of SEQ ID NO:78 is provided below:
MKPVTLYDVAEYAGVSYQTVSRVVNQASHVSAKTREKVEAAMAELNYIPNRVAQQ
LAGKQSLLIGVATSSLALHAPSQIVAAIKSRADQLGASVVVSMVERSGVEACKAAVH
NLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSDQTPINSIIFSHEDGTRLGV
EHLVALGHQQIALLAGPLSSVSARLRLAGWHKYLTRNQIQPIAEREGDWSAMSGFQ
QTMQMLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYDDTEDSSCYI
PPLTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNTQTASPR
ALADSLMQLARQVSRLESGQ (SEQ ID NO: 79)

[00325] In some embodiments, the nucleic acid comprises a *lacI* gene, wherein the *lacI* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 78. In some embodiments, the nucleic acid comprises a *lacI* gene, wherein the *lacI* gene comprises a nucleic acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the nucleic acid sequence of SEQ ID NO: 78.

[00326] In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a LacI protein, wherein said LacI protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 79. In some embodiments, the nucleic acid comprises a nucleic acid sequence encoding a LacI protein, wherein said LacI protein comprises an amino acid sequence that is at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the amino acid sequence of SEQ ID NO: 79.

[00327] In some embodiments, the nucleic acid comprises a *lacI* gene that is operably-linked to a regulatable promoter (*e.g.*, a sugar-regulatable promoter). In some embodiments, the nucleic acid comprises a *lacI* gene that is operably-linked to a sugar-regulatable promoter. In some embodiments, the sugar regulatable promoter exhibits increased activity (*e.g.*, increased transcription) in the presence of a specific sugar and decreased activity in the absence of a sugar. In some embodiments, the nucleic acid comprises a *lacI* gene that is operably-linked to a rhamnose-regulatable promoter (*e.g.*, a sugar-regulatable promoter). In some embodiments, the nucleic acid comprises a *lacI* gene that is operably-linked to an arabinose-regulatable promoter. In some embodiments, the

arabinose-regulatable promoter is P_{araBAD}. In some embodiments, the recombinant bacterium comprises the mutation Δ*relA*::*araC* P_{araBAD} *lacI* TT.

II. Pharmaceutical Compositions

[00328] A recombinant bacterium may be administered to a host as a pharmaceutical composition. In some embodiments, the pharmaceutical composition may be used as a vaccine to elicit an immune response to the recombinant bacterium, including any antigens that may be synthesized and delivered by the bacterium. In an exemplary embodiment, the immune response is protective. Immune responses to antigens are well studied and widely reported.

[00329] In some embodiments, the pharmaceutical composition comprises a recombinant bacterium described herein. In some embodiments, the pharmaceutical composition comprises a recombinant bacterium that synthesizes a *Salmonella* antigen of interest. In some embodiments, the pharmaceutical composition comprises a recombinant bacterium that synthesizes a *Campylobacter* antigen of interest. In some embodiments, the pharmaceutical composition comprises a recombinant bacterium that synthesizes a *Salmonella* antigen of interest and a recombinant bacterium that synthesizes a *Campylobacter* antigen of interest. In some embodiments, the pharmaceutical composition comprises a recombinant bacterium that synthesizes an antigen of interest comprising a *Campylobacter* N-glycan. In some embodiments, the pharmaceutical composition comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine or more recombinant bacterial strains, as described herein.

[00330] Pharmaceutical compositions may be administered to any host capable of mounting an immune response. Such hosts may include all vertebrates, for example, mammals, including domestic animals, agricultural animals, laboratory animals, and humans, and various species of birds, including domestic birds and birds of agricultural importance. Preferably, the host is a warm-blooded animal. In one embodiment, the host is a cow. In some embodiments, the host is an equine. In another embodiment, the host is an avian. In another embodiment, the host is a human.

[00331] The pharmaceutical composition can be administered to the subject as a prophylactic or for treatment purposes. In some embodiments, the pharmaceutical composition can be administered for the prophylaxis or treatment of salmonellosis. In some embodiments, the pharmaceutical composition can be administered for the prophylaxis or

treatment of a *Campylobacter* infection. In some embodiments, the pharmaceutical composition can be administered for the prophylaxis or treatment of salmonellosis and/or a *Campylobacter* infection.

[00332] In some embodiments, the recombinant bacterium is alive when administered to a host in a pharmaceutical composition described herein. Suitable vaccine composition formulations and methods of administration are detailed below.

[00333] A pharmaceutical composition comprising a recombinant bacterium may optionally comprise one or more possible additives, such as carriers, preservatives, stabilizers, adjuvants, and other substances.

[00334] In one embodiment, the pharmaceutical composition comprises an adjuvant. Adjuvants are optionally added to increase the ability of the vaccine to trigger, enhance, or prolong an immune response. In exemplary embodiments, the use of a live attenuated recombinant bacterium may act as a natural adjuvant. In some embodiments, the recombinant bacterium synthesizes and secretes an immune modulator. Additional materials, such as cytokines, chemokines, and bacterial nucleic acid sequences naturally found in bacteria, like CpG, are also potential vaccine adjuvants.

[00335] In some embodiments, the pharmaceutical composition comprises buffered saline (*e.g.,* phosphate-buffered saline (PBS)).

[00336] In some embodiments, the pharmaceutical composition comprises a food product.

[00337] In another embodiment, the pharmaceutical may comprise a pharmaceutical carrier (or excipient). Such a carrier may be any solvent or solid material for encapsulation that is non-toxic to the inoculated host and compatible with the recombinant bacterium. A carrier may give form or consistency, or act as a diluent. Suitable pharmaceutical carriers may include liquid carriers, such as normal saline and other non-toxic salts at or near physiological concentrations, and solid carriers not used for humans, such as talc or sucrose, or animal feed. Carriers may also include stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Carriers and excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington's Pharmaceutical Sciences 19th Ed. Mack Publishing (1995). When used for administering via the bronchial tubes, the pharmaceutical composition is preferably presented in the form of an aerosol.

[00338] In some embodiments, the pharmaceutical composition is delivered to a farm animal (*e.g.*, poultry). In some embodiments, the pharmaceutical composition is delivered as a course spray (*e.g.*, for use in hatcheries for delivery to poultry). In some embodiments, the pharmaceutical composition is delivered in the drinking water.

[00339] Care should be taken when using additives so that the live recombinant bacterium is not killed, or have its ability to effectively colonize lymphoid tissues such as the GALT, NALT and BALT compromised by the use of additives. Stabilizers, such as lactose or monosodium glutamate (MSG), may be added to stabilize the pharmaceutical composition against a variety of conditions, such as temperature variations or a freeze-drying process. The recombinant bacterium may also be co-administered with glutamate and/or arginite as described herein.

[00340] The dosages of a pharmaceutical composition can and will vary depending on the recombinant bacterium, the regulated antigen, and the intended host, as will be appreciated by one of skill in the art. Generally speaking, the dosage need only be sufficient to elicit a protective immune response in a majority of hosts. Routine experimentation may readily establish the required dosage. Typical initial dosages of vaccine for oral administration could be about 1 $x10^7$ to 1 x 10^{10} CFU depending upon the age of the host to be immunized. Administering multiple dosages may also be used as needed to provide the desired level of protective immunity.

[00341] In order to stimulate a preferred response of the GALT, NALT or BALT cells, administration of the pharmaceutical composition directly into the gut, nasopharynx, or bronchus is preferred, such as by oral administration, intranasal administration, gastric intubation or in the form of aerosols, although other methods of administering the recombinant bacterium, such as intravenous, intramuscular, subcutaneous injection or intramammary, intrapenial, intrarectal, vaginal administration, or other parenteral routes, are possible, *e.g.*, for anti-cancer applications.

[00342] In some embodiments, these compositions are formulated for administration by injection (*e.g.*, intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.).

[00343] In another embodiment, the disclosure provides a method for eliciting an immune response against an antigen in a host. The method comprises administering to the host an effective amount of a pharmaceutical composition comprising a recombinant bacterium described herein.

[00344] In still another embodiment, a recombinant bacterium may be used in a method for eliciting an immune response against a pathogen in an individual in need thereof. The method comprises administrating to the host an effective amount of a pharmaceutical composition comprising a recombinant bacterium as described herein. In a further embodiment, a recombinant bacterium described herein may be used in a method for ameliorating one or more symptoms of an infectious disease in a host in need thereof. The method comprises administering an effective amount of a pharmaceutical composition comprising a recombinant bacterium as described herein.

EXAMPLES

[00345] The present invention is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references, including literature references, issued patents, and published patent applications, as cited throughout this application are hereby expressly incorporated herein by reference. It should further be understood that the contents of all the figures and tables attached hereto are also expressly incorporated herein by reference.

Example 1: Background and Overview

[00346] *Campylobacter* is a leading cause of bacterial foodborne gastroenteritis worldwide and is a major public health problem (21-23). A recent estimate by the CDC indicates that *C. jejuni* is not only among the most common causes of foodborne illnesses in humans (over 800,000 cases per year), but also is a leading cause of hospitalization (over 8,000 annually) (24). Patients infected with *C. jejuni* often experience watery/bloody diarrhea, abdominal cramps, nausea, and fever. Severe neurological sequelae, bacteremia and other extraintestinal complications may develop infrequently (25). *C. jejuni* is widespread in food-producing animals, especially in poultry. The majority of human *C. jejuni* infections are predominantly associated with poor handling of raw chicken or consumption of undercooked chicken (1, 2, 26-32). The predominant role of poultry in human campylobacteriosis is supported by high prevalence of *C. jejuni* in both live birds and on carcasses, findings from epidemiological studies, and detection of identical genotypes in both poultry and human infections (28-30, 33, 34).

[00347] Domestic poultry including chickens, turkeys, ducks, and geese, are frequently infected with *C. jejuni* and *C. coli* (3, 35-37). Despite extensive colonization in the

intestinal tract, *Campylobacter* infection produces little or no clinical diseases in poultry (3, 35, 38, 39). Prevalence studies conducted in Europe and the U.S. have reported *C. jejuni*-positive flocks ranging from 2% to 100% (38, 40-42). Typically, the prevalence of *C. jejuni* increases as the birds grow and reaches highest points at the slaughter age for broilers. Once a broiler flock is infected with *C. jejuni*, the majority of birds within the flock become colonized in (40, 43-46). The high numbers of *C. jejuni* in the intestinal tract results in contamination of poultry carcasses during slaughter such that *C. jejuni* on poultry carcasses at the end of the processing line (post-chill) is usually over 50% varying from 0 to 100% (33, 47-53). In the U.S., several studies reported that a large percentage of processed broiler carcasses were contaminated with high numbers of *C. jejuni* (47, 53-55). Carcass contamination by *C. jejuni* is attributable to the farm of origin as high prevalence on a farm is usually associated with high-level carcass contamination in processing plants (56-60).

[00348] Salmonellosis develops different syndromes, including gastroenteritis, enteric fever (typhoid fever), and bacteremia, and as asymptomatic carriage in animals and humans (61). It is the leading cause of foodborne illness in the U.S., with 35% of the hospitalizations and 28% of the deaths (24). There are approximately 1.03 million cases of non-typhoidal *Salmonella* each year in the U.S., costing an economic loss of approximately $3.31 billion due to premature mortality, disability, and medical and productivity costs, with an annual loss of 16,782 quality-adjusted life years (62). *Salmonella* has a broad host range and adapts to survive in a wide range of different environments, even up to 16 months in dry feed stored at 25°C (63, 64). Although a large number of human infections are associated with food animal sources, infections also come from pets, reptiles, fruits, vegetables and other humans (65-67). Transmission of *Salmonella* to humans typically occurs when ingesting foods that are contaminated by animal feces or cross-contaminated by other sources (4). Among these sources, poultry and poultry-associated products are widely recognized as being among the most important vehicles for human *Salmonella* infections according to CDC reports (5, 67-74). With increasing consumption of poultry and poultry products, the number of salmonellosis associated with poultry continues to be a significant public health issue in the U.S.

[00349] There are over 2000 *Salmonella* serotypes. *S.* Enteritidis, *S.* Heidelberg, *S.* Kentucky and *S.* Typhimurium are commonly associated with chickens and to various extents with other food animal species and human infections. According to the CDC and USDA, *S.* Enteritidis was the most common serovar implicated in human illness in the

U.S. (74) and most commonly associated with chickens and eggs and to a much lesser extent with other food animal species (74-80). *S.* Heidelberg is also found in most major food animal species, eggs, and retail meat samples and is among the top five most common serotypes associated with human disease (79, 81, 82). Although, *S.* Kentucky rarely causes human infections in the United States, it is an emerging serovar in Europe and North Africa (83) and is prevalent in poultry (75, 84, 85).

[00350] Traditionally, antibiotics have been used to treat bacterial infections and for growth promotion in food animals. However, these uses of antibiotics contributed to increasing rates of antibiotic resistance (86), resulting in contamination of flocks and food products by antibiotic-resistant *Campylobacter*, *Salmonella*, *Enterococcus* and *Escherichia coli* and thereby increasing risks of human infections (87). Public concerns over the spread of antibiotic resistance in zoonotic bacterial pathogens, which poses a threat to the effectiveness of existing antibiotic therapy in both clinical medical and veterinary practice (88-97), led the European Union, in 1999, to ban use of most antibiotics for growth promotion to preserve the effectiveness of important human drugs (98). In 2004, the U.S. FDA banned enrofloxacin in food animals on the grounds that its use contributed to fluoroquinolone resistance in human pathogens. More recently, FDA and industry have agreed to cease use of growth promotion antimicrobials. However, there are concerns that reductions in antibiotic use in animal production may lead to an increase in foodborne pathogens on meat and other animal food. Besides management practices, it is necessary to develop other effective ways to mitigate emergence of antibiotic-resistant bacteria and to control foodborne pathogens. One of the best prevention strategies are development and use of vaccines.

[00351] ***Campylobacter* control:** Poultry houses can be contaminated by *Campylobacter* in many different ways from various environmental sources, making prevention of flock colonization by *Campylobacter* a very challenging task. In general, the on-farm control strategies examined for *Campylobacter* in poultry can be broadly divided into two approaches: 1) prevention of flock colonization by use of biosecurity-based interventions, and 2) prevention and/or reduction of *Campylobacter* colonization by non-biosecurity based measures such as vaccination, addition of bacteriocins, bacteriophages and feed additives, and competitive exclusion (42, 99-101). Improving biosecurity on farms apparently has a noticeable effect on lowering the overall flock prevalence. However, even the most stringent biosecurity measures do not always have a consistent and predictable effect on controlling *Campylobacter* and their effectiveness on flock prevalence is difficult to

assess under commercial settings (42, 102-105). In addition, stringent biosecurity measures are cost-prohibitive, hard to maintain, and their effectiveness seems to vary with production systems (36, 42). Multiple non-biosecurity measures have been evaluated for control of *Campylobacter* in live birds. Currently, there are no commercially available competitive exclusion products, vaccines, bacteriocins, bacteriophages or feed/water additives for excluding *Campylobacter* from chickens under production conditions although some promising results obtained under laboratory conditions have recently been reported (99, 106-108).

[00352] Vaccination against *C. jejuni* is a promising strategy but requires optimization of the vaccination regimen (e.g., induction of mucosal and possibly cellular immunity and practical delivery systems) using protective antigens. *C. jejuni* is generally considered as a commensal of chickens even though systemic and mucosal humoral responses have been observed (109-111) and anti-*Campylobacter* antibodies are detectable in progenies as maternally-derived antibodies (112). However, lack of strong activation of innate/adaptive immune responses in chickens, unlike in humans, requires development of vaccine strategies that enhance the immune response to *C. jejuni* antigens. For *C. jejuni* vaccine antigens to be immunogenic, it is critical to deliver antigens to the regional lymph tissues and spleen to stimulate effective mucosal immunity and systemic antibody and cellular immune responses. Therefore, *S.* Typhimurium-derived RASVs delivering *C. jejuni* protective antigens were constructed to control/reduce *C. jejuni* prevalence in poultry.

[00353] ***Salmonella* control.** Since contaminated poultry products are the major source of human *Salmonella* infection, vaccination of chickens is an important strategy to reduce the levels of *Salmonella* in poultry flocks, which will ultimately lead to lower rates of human *Salmonella* infection. Considering the whole life span of broilers as only 5-6 weeks, it is a challenge to develop a safe and effective *Salmonella* vaccine that could be cross-protective against different serovars. Currently, there are 3 types of vaccine, live attenuated, inactivated and subunit; only the former two are licensed for chickens. Live attenuated vaccines can be administered orally, delivering a bolus of antigens to the host and induce both antibody and cell-mediate immune responses. These live vaccines have used diverse attenuation strategies for strains of *S.* Typhimurium (113-115) and *S.* Enteritidis (115-121). However, variable efficacy and persistence, reversion to virulence, lack of cross protection and/or possible interference with *Salmonella* testing procedures are concerns (122-125). Killed vaccines are safe, but they must be delivered by costly injection and require

adjuvants to increase efficacy (123-125). Thus, the need for a safe and improved live attenuated but highly immunogenic *Salmonella* vaccine still exists (125) . *Salmonella* serotypes are defined by the immunologically heterogeneous LPS O-antigen side chains and flagellar antigens. Therefore, induction of immune responses to these heterogeneous antigens will not serve well the purpose of inducing cross-protective immunity to the diversity of serotypes. On the other hand, *Salmonella* and other enteric bacterial pathogens possess a number of immunologically related cross-reactive antigens. These include the LPS core polysaccharide which is the same in most, if not all, *S. enterica* serotypes except for *S. enterica* serotype Arizonae (126). In addition, numerous outer membrane proteins (OMPs), although possessing micro-heterogeneity, nevertheless share antigenic determinants (127) as do the iron regulated outer membrane proteins (IROMPs) that are required for iron acquisition (128), an essential important function for pathogen success within an infected animal. Strategies for *Salmonella* vaccines have been developed to display wild-type surface antigenic determinants *in vitro* and during the initial phase of infection through mucosal surfaces in the orally immunized host and then cease to synthesize LPS O-antigen side chains and to constitutively synthesize iron-regulated outer membrane proteins (IROMPs) in internal organs (19, 129). Gradual elimination of LPS O-antigen side chains *in vivo* also better exposes the immunologically related cross-reactive OMPs and IROMPs for surveillance by and stimulation of the immune system. Immune responses to IROMPs are known to be effective in preventing septicemic infection with enteropathogens, especially *E. coli* causing colisepticemia in chickens and turkeys (130). Also, antibodies induced to IROMPs from one bacterial serotype can recognize IROMPs synthesized by other serotypes (131). The *fur* gene encodes a repressor that, in the presence of free iron, represses all genes encoding IROMPs (128). When iron concentrations become low, as is the case in animal host tissues beyond the intestinal wall barrier, the Fur ceases to be synthesized at a high level and one observes constitutive expression of IROMPs and other Fur-regulated genes needed to sequester iron away from the infected animal host (128). *fur* mutants are attenuated when fed orally, giving a two to three log higher LD_{50} when administered either to mice (132) or day-of-hatch chicks (133). To achieve a high constitutive level of synthesis of all components for iron acquisition including IROMPs, the promoter of the *fur* gene (P_{fur}) was deleted and replaced with a tightly regulated *araC* P_{BAD} activator-promoter so that expression of the *fur* gene is solely dependent on the presence of arabinose (129) and is blind to the concentration of iron. Growth of *S.* Typhimurium vaccines with a ΔP_{fur}::TT *araC* P_{araBAD} *fur* deletion-insertion mutation in media

with a low level of arabinose results in very good colonization of lymphoid tissues, total attenuation at doses of 10^9 CFU and very high levels of induced protective immunity (129).

[00354] Genetically engineered *Salmonella* serovars Typhimurium (134), Paratyphi A and Typhi (135, 136) have been created as vaccines for regulated delayed attenuation *in vivo* (129, 134), regulated delayed *in vivo* synthesis of protective antigens specified by codon-optimized DNA sequences (137-140) and regulated delayed lysis *in vivo* (140-142) such that vaccines can be grown under conditions that enable them to display after course spray or oral immunization the capabilities of a wild-type strain to survive host defense stresses and efficiently colonize effector lymphoid tissues before manifesting attenuation to preclude disease symptoms and to synthesize protein antigens to induce protective immune responses. Strains have been engineered to eliminate or decrease synthesis of serotype-specific LPS O-antigen (18, 143) and flagellar antigens, to expose conserved LPS core (18, 19, 143) and over-express immunologically cross-reactive surface outer membrane protein antigens (19, 144) needed for the acquisition of essential iron and manganese ions, to diminish induction of gastroenteritis symptoms (145) while retaining abilities to recruit innate immunity, and to exhibit biological containment by cell lysis to preclude persistence *in vivo* or survival if excreted (140-142). Strains are totally safe at high doses to newborn (136, 146, 147), pregnant, protein malnourished and immunocompromised mice. Also neonatal pups (7 days old) born from mothers immunized with the same RASV strain develop better immune responses and exhibit higher levels of protective immunity to challenge than pups born from unimmunized mothers. A somewhat analogous result was observed years ago when chicks hatched from eggs laid by immunized hens displayed higher levels of protective immunity to the same attenuated *S.* Typhimurium vaccine used to immunize the hens than chicks from eggs laid by unimmunized hens (148). These technologies have been used to develop vaccines to prevent infections of newborns with *Streptococcus pneumoniae* (147), *Mycobacterium tuberculosis* (140, 149), a diversity of enteric bacterial pathogens causing diarrheal diseases in humans and influenza virus (141). In a recent human trial of a *S.* Typhi derived RASV, complete safety and shedding of zero viable vaccine cells in stools collected for 12 days was observed and with vaccine doses of up to 10^{10} CFU administered orally (150). Vaccines have also been developed using *S.* Typhimurium RASVs against pathogens of agriculturally important animals, especially to pathogens of chickens such as *Eimeria* species causing coccidiosis (151, 152) and *Clostridium perfringens* causing necrotic enteritis (153, 154). In these regards, six studies (140-142, 154-156) have demonstrated that RASVs

with the regulated delayed lysis *in vivo* phenotype gave superior levels of immune responses and protective immunity compared to RASVs that did not have this lysis phenotype.

[00355] To eliminate use of plasmid vectors with non-permitted drug resistance genes and to stabilize plasmid vectors in RASVs *in vivo*, (157) a balanced-lethal vector-host system was developed by deletion of the *asd* gene to impose an obligate requirement for diaminopimelic acid (DAP), an essential constituent of the rigid layer of the cell wall and a plasmid vector with the wild-type *asd* gene. It was also demonstrated that better immune responses to synthesized protective antigens could be achieved by having them secreted using T2SSs such as fusion to the N- and C-terminal β-lactamase sequences (158, 159), which has now been further optimized (154). Enhanced immunogenicity is partially due to enhanced formation of immunogenic outer membrane vesicles containing protective antigens (19, 160).

[00356] Plasmids were also constructed (118) and used that are required for the display of the regulated delayed lysis in vivo phenotype in conjunction with the derivatives of χ11730, χ11791 and χ12341. These strains have an *araC* P_{araBAD} regulated expression of the *murA* gene encoding the first enzyme in muramic acid synthesis and the Δ*asd* mutation blocking DAP synthesis in its chromosome. These lysis plasmids cause synthesis of protective antigens for delivery by cell lysis and have *araC* P_{araBAD} regulated *murA* and *asd* genes with GTG start codons to decrease translation efficiency. The P22 P_R located with opposite orientation to the transcription of the plasmid *araC* P_{araBAD} GTG-*murA* GTG-*asd* genes is repressed by the C2 repressor made during growth of the strain in medium with arabinose (due to the Δ*asdA*::TT *araC* P_{araBAD} *c2* mutation). However, C2 concentration decreases due to cell division *in vivo* to cause P_R-directed synthesis of anti-sense mRNA to block translation of residual *asdA* and *murA* mRNA. Transcription terminators (TT) flank all plasmid domains for controlled lysis, replication and gene expression so that expression in one domain does not affect activities of another domain.

A. Protective immunity against Eimeria infection in chickens.

[00357] Protective responses in chickens against *E. acervulina* causing coccidiosis was demonstrated. **Figs. 1A** and **1B** show comparative data for immunization of chickens with non-lysis and lysis strains without and with the Δ*sifA* mutation for delivery of the *Eimeria* SO7 antigen. The Δ*sifA* mutation enables the RASV to escape from the *Salmonella* containing vesicle (SCV) so that lysis occurs in the cytosol to enhance induction of CD8-, CD17- and NKT-dependent cellular immunities. Clearly, the most extensive *Eimeria* SO7-specific lymphocyte proliferation was induced by RASV strains with the lysis

phenotype and the Δ*sifA* mutation (Group 7). This same group gave the highest levels of antigen-specific IgA antibody responses (**Fig. 1C** and **1D**), CD8 cells (**Fig. 2A**), cytokine response (**Fig. 2B**), and weight gain after *Eimeria* oocyst challenge (**Table 1**). Based on these results, the Δ*sifA* mutation is included in the RASV vector strains to specify synthesis and delivery of *C. jejuni* protective antigens. These collective results demonstrate the efficacy in using these improved RASVs to develop vaccines that will confer protective immunity to *Salmonella* and *C. jejuni* strains in chickens. The use of RASVs that also exhibit complete biological containment with no persistence *in vivo* or survival if shed in feces, provides another important benefit.

Table 1. Immunization of broiler chickens with RASV strains and challenged with *E. tenella* oocysts

Group	[a]Primary Immunization	Secondary Immunization	[b]Oocyst challenge	[c]Weight gain (mean ± SEM)	[d]Feed conversion ratio (FCR)
1	BSG	BSG	No	576 ± 32	1.75 ± 0.17
2	BSG	BSG	Yes	510 ± 17	1.95 ± 0.06
3	χ11791(pYA3681)	Yes	Yes	491 ± 36	2.22 ± 0.47
4	χ11442(pYA5301)	Yes	Yes	502 ± 58	2.04 ± 0.17
5	χ11840(pYA5301)	Yes	Yes	433 ± 28	2.03 ± 0.19
6	χ11730(pYA5293)	Yes	Yes	558 ± 7	1.91 ± 0.08
7	χ11791(pYA5293)	Yes	Yes	598 ± 20	1.77 ± 0.00

[a]Chickens were orally vaccinated when 1 week old and again 1 week later.
[b]Three weeks later, groups G2 to G7 were inoculated with 10^5 *E. tenella* oocysts.
Group 3 = Empty Vector Control. Groups 3 & 4 = Non-Lysis RASVs delivering Eimeria S07 antigen. Groups 6 & 7 = Lysis RASVs delivering S07 antigen. Group 7 RASV also escapes from the *Salmonella*-containing vesicle for lysis in the cytosol to induce maximal systemic and cellular immunities.
[c]Weight gain in grams calculated over the infection period (6 days) between *E. tenella* challenge and termination.
[d]FCR, feed conversion ratio calculated as average amount of feed (g) consumed/average weight gain over the infection period (6 days) between *E. tenella* challenge and termination.

B. S. Typhimurium vaccine vector strains

[00358] *E. coli* host strains for construction of recombinant plasmid vectors and for conjugational transfer of suicide vectors for construction of *S.* Typhimurium with improved properties with added mutational alterations are listed in **Table 2**. Strains used for the construction and evaluation of RASV strains synthesizing and delivering the *Eimeria* SO7 antigen and *C. jejuni* antigens are also listed in **Table 2**. The phenotypic attributes associated with the mutations in these strains are described in **Table 3**.

Table 2: Bacterial Strains

***E. coli* strains**
χ6212(pYA232): φ80d *lacZ* ΔM15 *deoR* Δ(*lacZYA-argF*)*U169 supE44* λ^- *gyrA96 recA1 relA1 endA1* Δ*asdA4* Δ*zhf-2::*Tn*10 hsdR17* (r^-m^+), $lacI_q$ in pSC101 *ori*, Tc plasmid 10.2 kb
χ7213: *thi-1 thr-1 leuB6 glnV44 fhuA21 lacY1 recA1* RP4-2-Tc::Mu [λ*pir*] Δ*asdA4* Δ*zhf-2*::Tn*10*
***S.* Typhimurium strains**
χ11442: Δ*asdA33* Δ*relA198*::*araC* P_{BAD} *lacI* TT Δ*araBAD23* Δ*pmi-2426*
χ11730: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA198*::*araC* P_{araBAD} *lacI* TT
χ11791: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{BAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA198*::*araC* P_{araBAD} *lacI* TT Δ*sifA26*
χ11840: Δ*asdA33* Δ*relA198*::*araC* P_{araBAD} *lacI* TT Δ*araBAD23* Δ*pmi-2426* Δ*sifA26*
χ12341: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA197*::*araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL*
χ12396: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA197*::*araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL* ΔP_{fur33}::TT *araC* P_{araBAD} *fur*
χ12445: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA197*:: *araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*wbaP45* Δ*pagL14*::TT *araC* P_{araBAD} *wbaP*
χ12452: ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ(*wza-wcaM*)-*8* Δ*pmi-2426* Δ*relA197*::*araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL* Δ*ompA11* Δ*sopB1925*

Table 3. Mutations and associated phenotypes used in *S.* Typhimurium vaccine strains[a]

Genotype	Phenotype
A. Deletion and deletion-insertion mutations to confer a regulated delayed attenuation	
Δ*pmi*	encodes phosphomannose isomerase needed for synthesis of GDP-mannose for LPS O-antigen and thus necessary for virulence (18) .
Δ*waaL* Δ*pagL*::TT *rhaRS* P_{rhaBAD} *waaL*	regulates synthesis of enzyme responsible for attaching first subunit of LPS O-antigen to the LPS core (the deletion of the *waaL* gene is necessary to prevent impairment in expression of other *rfb* operon genes (161); the regulated expression cassette is therefore inserted into the *pagL* gene.
ΔP_{fur}::TT *araC* P_{BAD} *fur*	makes synthesis of Fur dependent on presence of arabinose in growth medium and ceases to be synthesized *in vivo* due to the absence of arabinose (162) (104). Fur decreases as a consequence of cell division *in vivo* to lead to elevated synthesis of IROMPs and attenuation presumably due to an iron overload.
ΔP_{mntR}::TT *araC* P_{BAD} *mntR*	makes synthesis of MntR dependent on presence of arabinose in growth medium and ceases to be synthesized *in vivo* due to the absence of

arabinose (162) (104). MntR decreases as a consequence of cell division *in vivo* to cause elevated synthesis of MnROMPs to enhance induction of cross-protective immunity.

B. Promoters and deletion-insertion mutations for regulated *in vivo* synthesis of antigens

P_{trc}	a promoter expressed at high level under both anaerobic and aerobic conditions and repressed by LacI (163).
Δ*relA*::*araC* P_{BAD} *lacI* TT	the *relA* mutation uncouples growth regulation from a dependence of protein synthesis, an important attribute in strains with regulated delayed lysis (164) (189). The arabinose-dependent synthesis of the LacI repressor is to enable a regulated delayed expression of DNA sequences under the control of P_{trc} (137) (113).
Phage P22 P_L and P_R	these promoters are repressible by the arabinose-dependent synthesis of the C2 repressor (Vander Byl and Kropinski (190) (165).

C. Deletion and deletion-insertion mutations to facilitate regulated delayed lysis *in vivo*

ΔP_{murA}::TT *araC* P_{BAD} *murA*	makes synthesis of MurA, the first enzyme in the synthesis of muramic acid, dependent on presence of arabinose in growth medium and ceases to be synthesized *in vivo* due to the absence of arabinose (142) (118). MurA decreases as a consequence of cell division *in vivo* to ultimately lead to cell lysis and death (166) (191). The *murA* defect is complemented by $MurA^+$ plasmid vectors (142) (118).
Δ*asdA*::TT *araC* P_{BAD} *c2*	the Asd enzyme is essential for the synthesis of diaminopimelic acid required for peptidoglycan synthesis (167) (192). The arabinose-dependent synthesis of the C2 repressor is to enable a regulated delayed expression of DNA sequences under the control of a promoter repressed by C2 (142) (118). The Δ*asdA* mutation is complemented by Asd^+ plasmid vectors (157) (193).
Δ(*wza-wcaM*)	eliminates twenty enzymes needed to synthesize several exopolysaccharides that promote biofilm formation and for synthesis of GDP-fucose that is required for colanic acid synthesis (168) (194), which can protect cells undergoing cell wall-less death from lysing (169) (195).

D. Mutations needed to enhance effective delivery of protective T-cell epitope antigens

Δ*sifA*	enables *Salmonella* to escape endosome = *Salmonella* containing vesicle (SCV) for lysis in cytosol (170) (196).

E. Other contributing mutations

Δ*recF*	eliminates recombinase that facilitates inter- and intra-plasmidic recombination (171-173) (197-199).
Δ*sopB*	eliminates excessive intestinal inflammation and enhances induction of mucosal immunity (145, 174-176)(122, 123)
Δ*ompA*	eliminates a major outer membrane protein that is highly immunogenic (177) but does not contribute to protective immunity (178)with elimination due to mutation leading to enhanced induction of protective immunity to other outer membrane proteins

[a] Δ = deletion; TT = transcription terminator; P = promoter

C. Campylobacter jejuni putative and demonstrated protective antigens.

[00359] Nineteen *C. jejuni* antigens were selected as known or probable protective antigens based on literature review and bioinformatics analyses. They are listed in **Table 4** as likely to induce protective immune responses.

Table 4. Exemplary *Campylobacter jejuni* antigens

Gene	Antigen	Function	GC contents	Signal peptide	bp/ aa	Identity among *C. jejuni* seq
cj0034c	Cj0034c	putative periplasmic protein	32.3%	22/23 for euk	702/233	96-100%
cj0113	Omp18	peptidoglycan associated lipoprotein	33.9	21/22 for euk	498/165	100%
cj0168c	Cj0168c	putative periplasmic protein	33.3	Yes 20/21	168/55	78-100%
cj0248	Cj0248	virulence factor and play a role in motility	30.8	No	858/285	90-100%
cj0289c	Peb3 AcfC	major antigenic peptide PEB3 accessory colonization factor	34.9	Yes 20/21	753/250	100%
cj0365c	CmeC	outer membrane channel protein CmeC (multidrug efflux system CmeAB	31.6	19/20 euk 23/24 grnN	1479/492	98-100%
cj0404	Cj0404	putative transmembrane protein	29.5	No	838/278	100%
cj0420	Cj0420	protein ycel precursor, polyisoprenoid-binding protein	28.8	21/22 gmN and euk	573/191	88-100%
cj0427	Cj0427	conserved hypothetical protein	34.5	No	336/111	99-100%
cj0428	Cj0428	hypothetical protein	33.1	No	384/127	82-100%
cj0588	TlyA	putative haemolysin	25.7	No	762/253	82-100%
cj0921c	Peb1	bifunctional adhesin/ABC transporter aspartate /glutamate-binding protein	31.7	Yes 26/27	780/259	100%
cj0982c	CjaA	surface antigen/glutamine ABC transporter substrate-binding protein	33.9	18/19 gmN/euk	840/279	100%
cj0998c	Cj0998c	Hypothetical protein	29.7	19/20 euk 20/21 gmN	573/190	100%
cj1259	Momp (PorA)	major outer membrane protein	36.1	Yes 22/23	1275/424	100%
cj1339c	FlaA	Flagellin A	37.0	No	1719/572	100%
cj1478c	CadF	outer membrane fibronectin-binding protein	31.8	16/17 euk very weak	960/319	100%
cj1534c	Dps	DNA starvation/stationary phase protection protein, putative bacterioferritin	32.0	No	450/149	93-100%
cj1656c	Cj1656c	putative motility protein	38.3	No	183/60	80-100%

cj sequences are from Campylobacter jejuni NCTC 11168 (ATCC700819),
* Analyzed using Blast against *C. jejuni* NCTC 11168 (ATCC700819), 327, LMG23211, 84-25, H693-13, CG8486, LMG9872, S3, LMG23269, 414, ATCC33560, 2008-831, 1997-4, 260.94. CF93-6, M1, RM221 & 20 additional sequenced *C. jejuni* genomes
euk: in eukaryotic; gmN: in gram negative

[00360] These sequences are present in all 39 *C. jejuni* genomes analyzed and have 82% or more amino acid sequence identity in all these strains. These include cj0034c, cj0113 (Omp18), cj0168c, cj0248, cj0289 (Peb3), cj0365 (CmeC), cj0404, cj0420, cj0427, cj0428, cj0588 (TlyA), cj0921c (Peb1), cj0982c (CjaA), cj0998c, cj1259 (PorA), cj1339c (Fla), cj1478c (CadF), cj1534c (Dps) and cj1656c. Each of the 19 antigens were analyzed using a diversity of bioinformatic analyses to determine which antigens are present in all sequenced *C. jejuni* strains, which are highly conserved in amino acid sequence, have signal sequences for export and are cell surface localized. Ten of these were selected, including: Cj0113 = Omp18 (Refs needed), Cj0289c = Peb3 (120-123), Cj0982c = CjaA (124-128), Cj1259 = PorA (120, 121, 123, 126, 129-134), Cj1339c = FlaA (120, 121, 123, 135-144), Cj1478 = CadF (120, 121, 129, 132, 144-147), Cj0588 TlyA (148-150), Cj0921c = Peb1 (121-123, 145, 151-157), Cj0998c (US 9,328,148 (150) and Cj1534c = Dps (158, 159) to generate recombinant plasmids using the regulated lysis plasmids pYA4763 and/or pG8R17 depending on whether the antigens did or did not have signal sequences for their secretion from *C. jejuni* strains.

[00361] The encoding sequences were codon optimized to adjust GC contents and optimize expression in *Salmonella* and also eliminate potential sites in mRNA subject to cleavage by RNase E so as to prolong half-life of mRNA to also enhance level of antigen synthesis. The codon optimized sequences were modified to delete the native signal peptide encoding sequence (if present), add a sequence encoding a His-tag at the C-terminal end to facilitate protein purification and other sequences on the N- and C-terminal ends to specify the nucleotide sequences to be recognized by restriction enzymes to enable cloning into either pYA4763 (if the antigen was not to be secreted by the RASV) (**Fig. 3A**) or into pG8R17 (**Fig. 3B**) with the much improved β-lactamase signal sequence (154)(131) if the antigen was to be secreted. The sequences were also designed to enable excision of the His-tag sequence at a later date, if desired. These sequences were synthesized commercially and returned in promoterless pUC *ori* vectors. The sequences to be cloned were excised from these plasmids and inserted into either of the lysis plasmids that were then electroporated into the *E. coli* strain χ6212(pYA232) (**Table 2**). pYA232 encodes the *lacI*^{q} gene that overproduces LacI to repress antigen synthesis by the recombinant strains in which the antigen-encoding sequences are under the control of P_{trc} that is repressed by LacI. After selection of the recombinant clones by selection for Asd^{+} and purification, viability is verified by ability of the *E. coli* recombinants to grow in the presence of IPTG to cause derepression and synthesis of the *C.*

jejuni recombinant antigens. Desirably growth is unaltered by antigen synthesis although in some cases growth is slightly retarded due to the high-levels of *C. jejuni* antigen synthesis. The recombinant lysis plasmids were then isolated from the χ6212 host strain and electroporated into the *S.* Typhimurium vaccine strain χ12341 (ΔP_{murA25}::TT *araC* P_{BAD} *murA* Δ*asdA27*::TT *araC* P_{BAD} *c2* Δ*pmi-2426* Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL* Δ(*wza-wcaM*)-*8* Δ*relA197*::*araC* P_{BAD} *lacI* TT Δ*recF126* Δ*sifA26*)) (**Table 2**) again selecting for Asd^+. After purification, RASVs were tested for rate of growth and synthesis of *C. jejuni a*ntigens with and without IPTG, for synthesis of LPS O-antigen dependent on growth in the presence of both mannose and rhamnose and for stability of plasmid maintenance and antigen synthesis after 50 generations of growth under permissive growth in media supplemented with DAP.

[00362] **Figs. 4A-4B** show western blot analyses of recombinant antigen synthesis in χ12341. The selected recombinant pG8R plasmid is indicated at the top of the Figure and also whether the plasmid was derived from pYA4763 or from pG8R17. Protein was detected using horse radish peroxidase tagged anti His-tag monoclonal antibodies. In some cases, such as with pG8R130 encoding the CadF antigen, the level of CadF protein is low indicating poor synthesis or degradation of the antigen. These problems are sometimes encountered and can be evaluated. Antigen synthesis is thus induced by exposure of RASV strains to IPTG for two hours and then adding 50 μg chloramphenicol;ml to block further protein synthesis. Samples are then taken every hour for four hours to measure antigen levels by western blot analyses. Maintenance of constant antigen levels rules out instability. If instability is observed the amino acid sequence is scrutinized with computer programs to indicate potential cleavage sites by proteases and then new constructs are made with nucleotide changes to eliminate such potential protease cleavage sites. If it appears that antigen synthesis is low, the sequence is examined and new constructs are made to fully establish the basis for the problem and ultimately generate recombinant clones with high-level stable synthesis of the recombinant antigens. In some cases this necessitates modification of the SD sequence to enhance ribosome binding or other alterations in the N-terminal coding sequence to enhance translation efficiency. Since the delivery of multiple recombinant *C. jejuni* antigens to poultry is examined to select those that reduce cecal titers of challenge strains by 100-fold or more, it is not likely that more than three or four antigens will be needed in the final vaccine. Thus, problems in getting high-level stable synthesis of all potential antigens might not be necessary.

[00363] In evaluating RASV constructs to induce protective immunity against *Salmonella* serotypes prevalent in poultry (**Table 5**), some of the recombinant plasmids encoding *C. jejuni* antigens were introduced into χ12452 that is a derivative of χ12341 with Δ*sopB* and Δ*ompA* mutations. pG8R86 encoding CjaA, pG8R88 encoding Omp18, pG8R89 encoding Peb1, pG8R90 encoding Cj0998c, pG8R102 encoding Peb3, pG8R128 encoding Dps and pG8R129 encoding TlyA have thus been introduced. All of these constructs yield good levels of antigen synthesis as shown in **Fig. 4.** The relative abilities of the χ12341 constructs are compared versus the χ12452 constructs to protect against infection with the selected *Salmonella* serotype strains (**Table 5**). These constructs are also being evaluated for protection to challenge with the *C. jejuni* strains also listed in **Table 5**. In order to determine the best combination of *C. jejuni* antigens to result in the largest reductions in *C. jejuni* following challenge infection of non-immunized versus immunized chickens, different combinations of strains each delivering a separate *C. jejuni* antigen are evaluated for additive benefits in reducing cecal titers of challenge strains. In several studies with other RASVs, very similar, if not identical, levels of protective immunity have been found when immunizing with a mixture of two RASVs delivering two different protective antigens, as was observed when a single RASV delivered both antigens.

Table 5. Strains used for challenge studies

Strain	Genotype or Phenotype	Reference
Salmonella		
S. Typhimurium UK-1 χ3761	Wild Type B, 1, 4, [5], 12: i:1,2	(179)
S. Enteritidis χ3550	Wild Type D1 1,9,12: g,m	(144)
S. Heidelberg χ3749	Wild Type B 1,4,[5],12:r:1,2:-	(180)
S. Montevideo NR35 χ3217	Wild Type C1 6,7,14,[54]:g,m,[p],s:[1,2,7]	(113)
S. Hadar NR14 χ3210	Wild Type C2 6,8:Z_{10}: e,n,x	(113)
S. Infantis NR29 χ3213	Wild Type C1 6,7,14:r:1,5	(113)
S. Newport NR90 χ3240	Wild Type C2 6,8,20:e,h:1,2	M. Rosenfeld, Borstel Institute
S. Kentucky χ11609	Wild Type C3 8,20:i:Z6	John Maurer, U of Georgia
C. jejuni 81–176	Human isolate, O:23/36	(181)
C. jejuni NCTC11168	Human isolate, serotype O:2	(182)
C. jejuni 81116 (NCTC11828)	Human and Chicken, Lab strain O:6	(183)
C. jejuni RM1221	Chicken isolate HS:53	(184, 185)
C. jejuni Chicken Isolate Mix	Chicken	(186)

[00364] This research enhances food safety by immunization of poultry with multiple *S.* Typhimurium-derived RASVs delivering multiple protective *C. jejuni* antigens to reduce colonization and persistence in chickens of *Salmonella* serotypes and *C. jejuni* strains

to reduce their transmission through the food chain to humans. It is expected the selected vaccine will also enhance poultry farming productivity and decrease use of antibiotics contributing to improved economics of poultry production. Immunization of breeders as well as broiler chicks with these RASVs should reduce, if not eventually eliminate, transmission of *Salmonella* and *C. jejuni* through the food chain to humans.

D. Materials and Methods

a. Bacterial strains, media and bacterial growth.

[00365] Attenuated *S.* Typhimurium UK-1 stain induces protective immunity to challenge with all *S.* Typhimurium strains whereas other *S.* Typhimurium strains attenuated with the same mutations often cannot induce protective immunity to other *S.* Typhimurium strains, and definitely not to the highly virulent UK-1 strain (187). Therefore a strain that displays maximal ability to cause disease is used as the parental strains for all attenuated derivative vaccine strains. LB broth and agar is used as complex media for propagation and plating of *Salmonella* (*see* 163(188, 189)). Purple broth (Difco), which is devoid of arabinose, mannose and rhamnose, and minimal salts medium and agar are also used (190) (164). Chrome azurol S (CAS) plates is used to evaluate synthesis of Fe acquisition siderophores. MacConkey agar with 0.5% lactose (Lac) and 0.1% arabinose (Ara), when necessary, is used to enumerate bacteria from chickens. Tetrathionate or selenite broth, with or without supplements, is used to enrich for *Salmonella* from cecal and intestinal contents, the bursa of Fabricius, liver and spleen. Bacterial growth is monitored spectrophotometrically and by plating for colony counts. Sequenced and well-characterized *C. jejuni* strains NCTC11168, 81-176, 81116 and RM221 and *C. jejuni* isolates from chickens (**Table 5**) are used in challenge studies. These strains are cultured microaerophilically (85% N_2, 10% CO_2, 5%O_2) on Mueller-Hinton (MH) medium at 42°C for 24 h. For *C. jejuni* isolation from chicken feces and organs, MH agar plates are supplemented with *Campylobacter* selective supplement (SR117E; Oxoid, Lenexa, KS). Bacterial strains for the challenge studies are listed in **Table 5**.

b. Molecular and genetic procedures.

[00366] Methods for DNA isolation, restriction enzyme digestion, DNA cloning and use of PCR for construction and verification of vectors are standard (*see* 172 (191)). DNA sequence analyses were performed at the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR). All oligonucleotide and/or gene segment syntheses were performed using codon optimization to enhance translational efficiency in

Salmonella and stabilize mRNA by eliminating RNase E cleavage sites to prolong mRNA half-life (192-194) (173-175). Since live vaccines cannot display antibiotic resistance, defined unmarked deletion mutations are generated with and without insertions using suicide vector technologies (*see* 176-181 Edwards *et al.* (1998) *Gene* 207: 149-57(195); Kaniga *et al.* (1998) *Infect. Immun.* 66: 5599-606(196); Maloy and Nunn (1981) *J. Bacteriol.* 145: 1110-2(197); Miller and Mekalanos (1988) *J. Bacteriol.* 170: 2575-83(198); Ried and Collmer (1987) *Gene* 57: 239-46(199); and Roland *et al.* (1999) *Avian Dis.* 43: 429-41)(200)). Suicide vectors having flanking sequences derived from the *S.* Typhimurium parent χ3761 for generation of all defined mutations listed in **Table 3** have been used. These mutations can be introduced using either phage P22HT*int* transduction (201, 202) (182, 183) of suicide vectors integrated into the deletion mutation in the parental *S.* Typhimurium strain followed by selection for sucrose resistance or by conjugational transfer of suicide vectors using standard methods (201, 202) (195, 203) (176, 185) with the suicide vector donor strains χ7213 (181). All strains constructed are given Chi numbers and stored at -80°C. Plasmid constructs will be evaluated by DNA sequencing and the ability to specify synthesis of *C. jejuni* proteins using gel electrophoresis and western blot analyses.

c. RASV strain characterization.

[00367] Vaccine strains have been fully characterized at each step in their construction and after introducing plasmids encoding *C. jejuni* antigens. Initially, the *C. jejuni* antigens Peb1 and Omp18 will be evaluated to enable comparative analyses of the different RASV vector strains χ12341 and χ12452. Then, we will evaluate different combinations of two strains to establish a rank order in giving the largest decrease in cecal titers of the *C. jejuni* challenge strains. Prior to commencing immunization studies, RASVs have been compared with vector control strains for stability of plasmid maintenance, integrity, and antigen synthesis ability when grown in the presence of arabinose and DAP for 50 generations. For constitutive synthesis of the *C. jejuni* antigens, strains are grown in the presence of IPTG to determine whether it results in poor growth and/or genetic instability. Molecular genetic attributes have been confirmed by PCR and/or Southern blot analyses. Measurement of LPS core and O-antigen has been performed after electrophoresis using silver-stained gels (204) (186). This analysis is performed after every step in any strain construction to eliminate rough variants should they arise. Motility tests and use of specific antisera for flagellar antigens are used to determine the presence of flagella. Final RASVs

have been assessed for bile sensitivity, acid tolerance, and the ability to survive in sera with and without complement inactivation (104,111). Complete sensitivity of the RASVs to antibiotics will also be evaluated. Metabolic attributes of vaccine strains are evaluated using API-20E tests.

d. Animal experimentation.

[00368] Testing of RASV strains: Studies on RASVs will be conducted in SPAFAS white leghorn chicks and in Cobb and/or Ross broiler chicks obtained from a commercial hatchery. Newly hatched white leghorn chicks will be immunized orally after which food and water will be provided *ad libitum*. Broiler chicks will be similarly orally immunized one day after receipt from the hatchery to enable them to acclimate but before feeding. Food will be provided after immunization. Vaccine strains to be evaluated (as well as *Salmonella* challenge strains) will be grown in LB broth to an OD_{600} of ~0.9, sedimented by centrifugation at room temperatures and suspended in PBS at densities of 5×10^{10} CFU/ml to enable oral doses of up to 1×10^{9} CFU to be administered in 20 µl into chicks. Final RASVs will be evaluated for induction of immune responses that diminish tissue (bursa, liver and spleen) and cecal titers of *Salmonella* serotype challenge strains and cecal titers of *C. jejuni* challenge strains. RASVs will be quantitatively enumerated in various tissues as a function of time after oral inoculation.

[00369] The NIH RAC and the UF IBC reclassified the RASV strains with the regulated lysis phenotype and complete biological containment for use at level 1 containment, in commercial settings and in outpatients, with initial immunizations in chicks housed in batteries or on litter. However, for challenge with *Salmonella* serotypes, birds are housed in Federal Design Molded Isolators. All experimental work is conducted in compliance with the regulations and policies of the Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the UF IACUC.

e. Statistical analysis.

[00370] All results are analyzed using the most appropriate statistical test from the SAS program to evaluate the relative significance or lack thereof of results obtained. In specific cases, the staff at the Clinical Translational Science Institute at UF is consulted to provide help with experimental design and statistical analyses services for animal and human studies and trials. Using ten chickens/treatment group will yield a statistical power > 0.80. In

previous studies it was found that n=10 is an adequate number of birds to obtain statistically significant differences between treatment groups. However, the best RASVs will be evaluated multiple times at two institutions to substantiate conclusions.

Example 2. Construction of RASV Strains

[00371] The starting strain for the development of derivative strains was *S.* Typhimurium χ12341 (ΔP$_{murA25}$::TT *araC* P$_{BAD}$ *murA* Δ*asdA27*::TT *araC* P$_{BAD}$ *c2* Δ*pmi-2426* Δ*waaL46* Δ*pagL64*::TT *rhaRS* P$_{rhaBAD}$ *waaL* Δ(*wza-wcaM*)-*8* Δ*relA197*::*araC* P$_{BAD}$ *lacI* TT Δ*recF126* Δ*sifA2*). This strain was derived in many steps from the *S.* Typhimurium UK-1 parent strain χ3761. Since live vaccines cannot display antibiotic resistance, defined unmarked deletion mutations were generated using suicide vector technologies (*see* Edwards *et al.* (1998) *Gene* 207: 149-57(195); Kaniga *et al.* (1998) *Infect. Immun.* 66: 5599-606(196); Maloy and Nunn (1981) *J. Bacteriol.* 145: 1110-2(197); Miller and Mekalanos (1988) *J. Bacteriol.* 170: 2575-83(198); Ried and Collmer (1987) *Gene* 57: 239-46(199); and Roland *et al.* (1999) *Avian Dis.* 43: 429-41)(200). Precise deletion and deletion-insertion mutations without interference on adjacent gene functions and without leaving repetitive scars that can contribute to genetic instability can be generated using a combination of molecular genetics, suicide vectors and phage P22-mediated transduction (Kang *et al.* (2002) *J. Bacteriol.* 184: 307-12)(205). These procedures were used to introduce the deletion and deletion-insertion mutations listed in **Table 3** to construct the strains listed in Table 2 as well as the derivatives described herein into *S.* Typhimurium χ12341. Suicide vectors derived from either pRE112 or pMEG-375 have been and are constructed, as necessary. To prevent an adverse effect by an inserted sequence on the expression of any adjacent genes, strong transcription terminator (TT) isolated from a bacteriophage genome can be inserted in appropriate locations to block such undesirable effects. These sequences can be inserted into the suicide vectors as appropriate for the individual constructions. Upon construction of suicide vectors and creating deletion and deletion-insertion mutations, allele numbers and Chi numbers are assigned to enable distinction. Strains are stored at -80°C.

Construction of RASV displaying the conserved C. jejuni N-glycan.

[00372] In this case, the 14 gene *C. jejuni pgl* operon encoding for synthesis of the highly conserved, cross-protective N-glycan (15, 206) also possessing adjuvant properties that is ligated to and transported by the universal UndPP carrier with attachment to the LPS

core will be inserted into a deletion of the *cysG* gene which is dispensable and a very useful site for insertion of foreign genes. Expression of the *pgl* operon will be driven by fusion to a LacI-regulatable P_{trc} modified by inclusion of a 35 bp sequence found in promoters of operons encoding many genes involved in polysaccharide synthesis (207, 208), which enables synthesis of long mRNA transcripts. This insertion into Δ*cysG* will be introduced into the χ12341 derivative χ12445 (**Table 2**) in which the Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL* mutations have been eliminated since the activity of WaaL is needed to cause ligation and transport of the *C. jejuni* N-glycan (209, 210). Since regulated cessation of the LPS O-antigen in vivo is desired to better display protective surface antigens, a deletion of the *wbaP* gene that encodes the enzyme that couples the first sugar galactose of the O-antigen repeat onto the LPS core and an *araC* P_{araBAD} *wbaP* into the *pagL* gene are present in χ12445. Thus, the *waaL* gene is expressed continuously and the WaaL enzyme will be able to ligate the *C. jejuni* N-glycan onto the LPS core, the synthesized Cj1433c protein and any *C. jejuni* or RASV surface proteins such as OmpC and OmpD with the D/E-X_1-N-X_2-T/S sequences that serve as the receptor sequence for N-glycosylation.

[00373] This χ12445 strain is further modified by insertion of all or part of the *cj1433c* gene (209) into a deletion of the *ompA* gene to generate a fusion of the Cj1433c protein, which has nine repeats of a DLNNT sequence for N-glycan binding, with the *ompA*-encoded signal sequence. It should be noted that the OmpA protein is one of the most abundant *Salmonella* surface antigens, is totally unnecessary for colonization and virulence and yet is one of the most immunogenic *Salmonella* proteins but unable to afford any protective immunity.

Example 3: RASV displaying *C. jejuni* N-glycan

[00374] One RASV currently being constructed will display the conserved *C. jejuni*-specific N-glycan antigen and synthesize a *C. jejuni* outer membrane protein with multiple targets for N-glycosylation. This will be achieved by expression of the 14-gene *pgl* operon from *C. jejuni* that encodes the enzymes to synthesize the immuno-protective N-linked glycan attached to many *C. jejuni* surface proteins on a balanced-lethal plasmid or after insertion into the chromosome and inserting sequences encoding the canonical bacterial N-glycosylation sequence D/E-X-N-Y-T/S in genes encoding RASV surface antigens. This RASV can be further modified to cause in vivo display of conserved cross-reactive antigens

for iron and manganese uptake to yield a RASV that will induce cross protective immunity to most *Salmonella* serotypes while also delivering multiple *C. jejuni* protein antigens to induce superior protective immunity against *C. jejuni*.

Construction of RASV encoding the C. jejuni pgl operon.

[00375] *C. jejuni* possesses a 14-gene *pgl* operon encoding enzymes for the synthesis and placement of a highly conserved, cross-protective N-glycan (15, 206). **Fig. 7** depicts the *pgl* operon. The N-glycan possesses adjuvant properties when ligated to and transported by the universal UndPP carrier for attachment to the LPS core. Due to the length of the *pgl* operon nucleotide sequence, no attempt has yet been made to optimize codons for expression in *Salmonella* since this would require codon-optimization and synthesis of segments that would then need to be reassembled into a full-length operon. Although this is possible, the native *C. jejuni* sequence has been initially cloned and inserted into a modified derivative (pG8R160) of the low copy number Asd^+ plasmid pYA3337 (pSC101 *ori*) that possesses the LacI repressible P_{trc} promotor modified by insertion of a 35 bp insertion that enhances complete transcription of long operons (206, 207). Since the *pglB* gene encodes an oligo polysaccharide transferase, it has a duplicate competing activity to the *Salmonella* WaaL enzyme and deletion of the *pglB* gene enabled better addition of the N-glycan to the LPS core (see **Fig. 5**) to enable its transfer to D/E-X-N-Y-T/S sequences in proteins. The *pglB* gene in the pG8R160-derived construct pG8R161 was therefore deleted. The inserted *pgl* operon sequence, which follows, has a ~30% GC content and still contains codons infrequently used in highly-expressed *Salmonella* genes. However, this may be beneficial for the well-being of the RASV derivative of χ12445 (ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ*pmi-2426* Δ(*wza-wcaM*)*-8* Δ*relA197*::*araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*wbaP45* Δ*pagL14*::TT *araC* P_{araBAD} *wbaP*) since over expression of genes even on a low copy number plasmid can result in decreased growth. Thus lower levels of gene expression are likely beneficial.

Construction of RASV displaying the conserved C. jejuni N-glycan.

[00376] The *pgl* operon has been inserted into the low copy number pYA3337 (pSC101 *ori*) Asd^+ plasmid pG8R161 (see above) and will be inserted into a deletion of the *cysG* gene, which is dispensable, and a very useful site for insertion of foreign genes. Expression of the *pgl* operon is driven by fusion to a LacI-regulatable P_{trc} promoter modified by inclusion of a 35 bp sequence found in promoters of operons encoding many genes involved in polysaccharide synthesis (207, 208). This enables synthesis of long mRNA

transcripts. The insertion into Δ*cysG* will be introduced into χ12445 (**Table 2**) in which the Δ*waaL46* Δ*pagL64*::TT *rhaRS* P_{rhaBAD} *waaL* mutations have been eliminated since the activity of WaaL is needed to cause ligation and transport of the *C. jejuni* N-glycan (209, 210). Since regulated cessation of LPS O-antigen synthesis in vivo to better display protective surface antigens, is desired, a deletion of the *wbaP* gene that encodes the enzyme that couples the first sugar galactose of the O-antigen repeat onto the LPS core was inserted, and an *araC* P_{araBAD} *wbaP* was inserted into the *pagL* gene. The resulting strain derived in multiple steps from χ12341 is χ12445 (ΔP_{murA25}::TT *araC* P_{araBAD} *murA* Δ*asdA27*::TT *araC* P_{araBAD} *c2* Δ*pmi-2426* Δ(*wza-wcaM*)*-8* Δ*relA197*::*araC* P_{araBAD} *lacI* TT Δ*recF126* Δ*sifA26* Δ*wbaP45* Δ*pagL14*::TT *araC* P_{araBAD} *wbaP*). In this strain either with a plasmid or the *cysG* insertion of the *pgl* operon, the *waaL* gene is expressed continuously and the WaaL enzyme will ligate the *C. jejuni* N-glycan onto the LPS core, the synthesized Cj1433c protein fusion (see below) and any *C. jejuni* or RASV surface proteins such as OmpC and OmpD with the D/E-X_1-N-X_2-T/S sequence that serves as the receptor sequence for N-glycosylation. χ12445 is further modified by insertion of the *Cj1433c* gene (209) into a partial deletion of the *ompA* gene to generate a fusion of the Cj1433c protein, which has nine repeats of a DLNNT sequence for N-glycan binding, with the *ompA*-encoded signal sequence. It should be noted that the OmpA protein is one of the most abundant *Salmonella* surface antigens, is totally unnecessary for colonization and virulence and yet is one of the most immunogenic *Salmonella* proteins but unable to afford any protective immunity. If desired, the ΔP_{fur}::TT *araC* P_{BAD} *fur* and ΔP_{mntR}::TT *araC* P_{BAD} *mntR* deletion-insertion mutations can be added to χ12445 to lead to the up-regulation in vivo of all genes needed for iron and manganese uptake. Since immune responses to IROMPs are cross reactive in the *Enterobacteriaceae*, this strain, as LPS O-antigen synthesis ceases in vivo (due to the *pmi* mutation and absence of mannose and the *araC* P_{araBAD} regulated shutoff of the *wbaP* gene) to better expose surface protein antigens, will induce enhanced cross-protective immunity to *Salmonella* serotypes and other enteric bacteria.

Construction of Cj1433c-OmpA fusion and insertion into the ΔompA11 mutation in χ12445.

[00377] The genome of *C. jejuni* is AT rich, which causes problems in transcription fidelity in *S.* Typhimurium. The Cj1433c gene is particularly 'bad' in that it has a GC content of only 26.4 %. Two successive codon optimizations to improve faithful

expression of this gene or parts of it in RASV strains were performed. The original and improved nucleotide sequences for *Cj14433c* gene are given below with indication of the sequences specifying N-glycosylation sites in the protein.

Cj1433c 1107 bp (368 aa) No signal peptide, GC of 26.4%
Identity among various *C. jejuni*; low (31%-100%) many sequences aligned only with partial sequence

1st line; original (26.4%)
2nd line; best optimized (45.9 %)
3rd lime; modified for higher GC (49.1%) serine, valine, glycine (S, V and G) modified
The N-glycan addition sites are underlined

TATAATATTTTTGGCAGTTTGTTTTAA AGGATGTTTTA

```
1/1                                                 31/11
ATG CAG CGA TTT AAA AAA TGG TTT TTG TCT ATC ATA AAA AAT TTC AAG CAA CAT GAA AAA
ATG CAG CGT TTC AAA AAA TGG TTC CTG TCT ATC ATC AAA AAC TTC AAA CAG CAC GAA AAA
ATG CAG CGT TTC AAA AAA TGG TTC CTG TCC ATC ATC AAA AAC TTC AAA CAG CAC GAA AAA
M   Q   R   F   K   K   W   F   L   S   I   I   K   N   F   K   Q   H   E   K

61/21                                               91/31
ATT AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT CTT AAT
ATC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC
ATC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC
I   K   I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   D   L   N

121/41                                              151/51
AAT ACA AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT CTT
AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG
AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG
N   T   K   I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   D   L

181/61                                              211/71
AAT AAT ACA AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT CTT AAT AAT ACA AAG ATA GAT
AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC
AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC
N   N   T   K   I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   D

241/81                                              271/91
CTT AAT AAT ACA AAG ATA GAA TTA TCG CAA TTA AAA AAA GAG CAC TAT AAA GTA TTA GAT
CTG AAC AAC ACC AAA ATC GAA CTG TCT CAG CTG AAA AAA GAA CAC TAC AAA GTT CTG GAC
CTG AAC AAC ACC AAA ATC GAA CTG TCC CAG CTG AAA AAA GAA CAC TAC AAA GTG CTG GAC
L   N   N   T   K   I   E   L   S   Q   L   K   K   E   H   Y   K   V   L   D

301/101                                             331/111
TTT CAT TTA AGA AAA ATT ACA CCT CAA GCT TTT TTA GAG ATT GTT GAA ATT CAT TTA GCC
TTC CAC CTG CGT AAA ATC ACC CCG CAG GCG TTC CTG GAA ATC GTT GAA ATC CAC CTG GCG
TTC CAC CTG CGT AAA ATC ACC CCG CAG GCG TTC CTG GAA ATC GTG GAA ATC CAC CTG GCG
F   H   L   R   K   I   T   P   Q   A   F   L   E   I   V   E   I   H   L   A

361/121                                             391/131
GAA TCA TGT AAT TTA AAT TGT TTT GGT TGT AAT CAT TTT TCA CAA ATA GCT GAA AAA GAA
GAA TCT TGC AAC CTG AAC TGC TTC GGT TGC AAC CAC TTC TCT CAG ATC GCG GAA AAA GAA
GAA TCC TGC AAC CTG AAC TGC TTC GGC TGC AAC CAC TTC TCC CAG ATC GCG GAA AAA GAA
E   S   C   N   L   N   C   F   G   C   N   H   F   S   Q   I   A   E   K   E

421/141                                             451/151
TTT CCA GAT ATA GAA ATT TTT AAA AAA GAT ATG CAA AGA CTT TCA GAA ATA TCT AAA GGT
```

```
TTC CCG GAC ATC GAA ATC TTC AAA AAA GAC ATG CAG CGT CTG TCT GAA ATC TCT AAA GGT
TTC CCG GAC ATC GAA ATC TTC AAA AAA GAC ATG CAG CGT CTG TCC GAA ATC TCT AAA GGC
F   P   D   I   E   I   F   K   K   D   M   Q   R   L   S   E   I   S   K   G

481/161                                                 511/171
ATT GTC GGA ACT TTT AGA TTG ATG GGT GGC GAA CCT CTT TTA AAT CCC AAT TGT ATA CAG
ATC GTT GGT ACC TTC CGT CTG ATG GGT GGT GAA CCG CTG CTG AAC CCG AAC TGC ATC CAG
ATC GTG GGC ACC TTC CGT CTG ATG GGC GGC GAA CCG CTG CTG AAC CCG AAC TGC ATC CAG
I   V   G   T   F   R   L   M   G   G   E   P   L   L   N   P   N   C   I   Q

541/181                                                 571/191
TTT TTT GAC ATT ACA AGA TAT TTT TTT CCA AAA AGT GCC ATT TGG TTG GTA ACT AAT GGT
TTC TTC GAC ATC ACC CGT TAC TTC TTC CCG AAA TCT GCG ATC TGG CTG GTT ACC AAC GGT
TTC TTC GAC ATC ACC CGT TAC TTC TTC CCG AAA TCC GCG ATC TGG CTG GTG ACC AAC GGC
F   F   D   I   T   R   Y   F   F   P   K   S   A   I   W   L   V   T   N   G

601/201                                                 631/211
ATT TTA CTT GAT AAG CAA AAT GAG GAT TTT TGG AAT TCA TGC CAA AGG AAT AAA ATG CAA
ATC CTG CTG GAC AAA CAG AAC GAA GAC TTC TGG AAC TCT TGC CAG CGT AAC AAA ATG CAG
ATC CTG CTG GAC AAA CAG AAC GAA GAC TTC TGG AAC TCC TGC CAG CGT AAC AAA ATG CAG
I   L   L   D   K   Q   N   E   D   F   W   N   S   C   Q   R   N   K   M   Q

661/221                                                 691/231
ATT CGT CCA ACA AAG TAT CCT ATA AAA ATT AAT TGG GAT TTG ATT AAA GAT AAG TGT GAT
ATC CGT CCG ACC AAA TAC CCG ATC AAA ATC AAC TGG GAC CTG ATC AAA GAC AAA TGC GAC
ATC CGT CCG ACC AAA TAC CCG ATC AAA ATC AAC TGG GAC CTG ATC AAA GAC AAA TGC GAC
I   R   P   T   K   Y   P   I   K   I   N   W   D   L   I   K   D   K   C   D

721/241                                                 751/251
CAA TAT GAT ATC CCC TTG ATA TTT TTT AAC AAT GGA GAG TTG GAA AAA ACT TCT TGG AAA
CAG TAC GAC ATC CCG CTG ATC TTC TTC AAC AAC GGT GAA CTG GAA AAA ACC TCT TGG AAA
CAG TAC GAC ATC CCG CTG ATC TTC TTC AAC AAC GGC GAA CTG GAA AAA ACC TCC TGG AAA
Q   Y   D   I   P   L   I   F   F   N   N   G   E   L   E   K   T   S   W   K

781/261                                                 811/271
TTT TCT CTA GAT CCT TCT GGA AAT TGT GAT AAT TAC CAT AGT TTT ACA AAT TGT AGT ATG
TTC TCT CTG GAC CCG TCT GGT AAC TGC GAC AAC TAC CAC TCT TTC ACC AAC TGC TCT ATG
TTC TCC CTG GAC CCG TCC GGC AAC TGC GAC AAC TAC CAC TCC TTC ACC AAC TGC TCC ATG
F   S   L   D   P   S   G   N   C   D   N   Y   H   S   F   T   N   C   S   M

841/281                                                 871/291
GCA AAT CAC TGT GTT CAG TTT AAA GAT GGA AAG CTA TTT ACT TGT ACC TTT CCT GCA CAT
GCG AAC CAC TGC GTT CAG TTC AAA GAC GGT AAA CTG TTC ACC TGC ACC TTC CCG GCG CAC
GCG AAC CAC TGC GTG CAG TTC AAA GAC GGC AAA CTG TTC ACC TGC ACC TTC CCG GCG CAC
A   N   H   C   V   Q   F   K   D   G   K   L   F   T   C   T   F   P   A   H

901/301                                                 931/311
GTA CAG CAT TTT AAT AAA AAG TAT GGA AAT CAT TTT GAA GTT TGC GAA TTT GAC TTT ATT
GTT CAG CAC TTC AAC AAA AAA TAC GGT AAC CAC TTC GAA GTT TGC GAA TTC GAC TTC ATC
GTG CAG CAC TTC AAC AAA AAA TAC GGC AAC CAC TTC GAA GTG TGC GAA TTC GAC TTC ATC
V   Q   H   F   N   K   K   Y   G   N   H   F   E   V   C   E   F   D   F   I

961/321                                                 991/331
GAT ATT TAT AAA GCC AAG GAT TAT CAA GAA ATT TTA TTT TTT CTT TCT AAG CCT ATT CCT
GAC ATC TAC AAA GCG AAA GAC TAC CAG GAA ATC CTG TTC TTC CTG TCT AAA CCG ATC CCG
GAC ATC TAC AAA GCG AAA GAC TAC CAG GAA ATC CTG TTC TTC CTG TCC AAA CCG ATC CCG
D   I   Y   K   A   K   D   Y   Q   E   I   L   F   F   L   S   K   P   I   P

1021/341                                                1051/351
TTT TGT AGA TAT TGC AAG GTA TCA CAA TGG GCA GAA ATT GGA AAA TGG CGT TCT AGC AAT
TTC TGC CGA TAC TGC AAA GTT TCT CAG TGG GCG GAA ATC GGT AAA TGG CGT TCT TCT AAC
TTC TGC CGA TAC TGC AAA GTG TCC CAG TGG GCG GAA ATC GGC AAA TGG CGT TCC TCC AAC
F   C   R   Y   C   K   V   S   Q   W   A   E   I   G   K   W   R   S   S   N

1081/361
AAA ACA AAA CAT GAA TAT TTA ATT TGA (SEQ ID NO:71 )
AAA ACC AAA CAC GAA TAC CTG ATC TGA (SEQ ID NO:72 )
AAA ACG AAA CAC GAA TAC CTG ATC TGA (SEQ ID NO:73 )
```

K T K H E Y L I * (SEQ ID NO:74)

[00378] The topology of the OmpA protein indicating all 16 transmembrane sequences was diagramed by Reusch et al., Int. J. Mol. Sci. 2013, 14(6): 10727-10748 (213)(214, 215), the entire contents of which are expressly incorporated herein by reference. Based on bioinformatic analyses, a portion of the *C. jejuni* Cj1433c gene encoding the repeats of the N-glycosylation sites (amino acids 21 to 86) was inserted as a substitute for the loop spanning and including the 3 and 4 transmembrane sequences (amino acids 70 to 107) of OmpA.

Encoding sequence for modified OmpA with insertion of portion of Cj1433c sequence

[00379] Beta loops 3 and 4 of OmpA (aa 70-107) are deleted and 66 aa (aa21 to 86) of Cj1433c are inserted that specify the nine repeats for N-glycosylation.

```
1/1                                                 31/11
ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC GCT ACC GTA GCG CAG
  M   K   K   T   A   I   A   I   A   V   A   L   A   G   F   A   T   V   A   Q

61/21                                               91/31
GCC GCT CCG AAA GAT AAC ACC TGG TAC GCT GGT GCT AAA CTG GGC TGG TCT CAG TAC CAT
A   A   P   K   D   N   T   W   Y   A   G   A   K   L   G   W   S   Q   Y   H

121/41                                              151/51
GAC ACC GGC TTC ATT CAC AAT GAT GGC CCG ACT CAT GAA AAC CAA CTG GGC GCA GGT GCT
  D   T   G   F   I   H   N   D   G   P   T   H   E   N   Q   L   G   A   G   A

181/61                                              211/71
TTT GGT GGT TAC CAG GTT AAC CCG TAT gtc gac ATC AAA ATC GAC CTG AAC AAC ACC AAA
F   G   G   Y   Q   V   N   P   Y   V   D   I   K   I   D   L   N   N   T   K
                            OmpA aa69                                Cj1433 aa21-86
241/81                                              271/91
ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC
  I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   D   L   N   N   T

301/101                                             331/111
AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC
K   I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   D   L   N   N

361/121                                             391/131
ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC GAC CTG AAC AAC ACC AAA ATC ctg cag ATC
  T   K   I   D   L   N   N   T   K   I   D   L   N   N   T   K   I   L   Q   I
                                                                    OmpA aa108
421/141                                             451/151
ACT GAC GAT CTG GAC GTT TAT ACC CGT CTG GGT GGT ATG GTA TGG CGT GCA GAC ACC AAG
T   D   D   L   D   V   Y   T   R   L   G   G   M   V   W   R   A   D   T   K
481/161                                             511/171
TCT AAC GTC CCT GGC GGC CCG TCT ACT AAA GAC CAC GAC ACC GGC GTT TCC CCG GTA TTC
  541/181                                             571/191
S   N   V   P   G   G   P   S   T   K   D   H   D   T   G   V   S   P   V   F
  GCG GGC GGT ATC GAG TAT GCT ATC ACC CCT GAA ATC GCA ACC CGT CTG GAA TAC CAG
  TGG
A   G   G   I   E   Y   A   I   T   P   E   I   A   T   R   L   E   Y   Q   W
601/201                                             631/211
ACT AAC AAC ATC GGT GAT GCC AAC ACC ATC GGC ACC CGT CCG GAC AAC GGC CTG CTG AGC
  T   N   N   I   G   D   A   N   T   I   G   T   R   P   D   N   G   L   L   S
  661/221                                             691/231
GTA GGT GTT TCC TAC CGT TTC GGC CAG CAA GAA GCT GCT CCG GTA GTA GCT CCG GCA CCA
V   G   V   S   Y   R   F   G   Q   Q   E   A   A   P   V   V   A   P   A   P
```

```
721/241                                          751/251
GCT CCG GCT CCG GAA GTA CAG ACC AAG CAC TTC ACT CTG AAG TCT GAC GTA CTG TTC AAC
  A   P   A   P   E   V   Q   T   K   H   F   T   L   K   S   D   V   L   F   N
  781/261                                          811/271
TTC AAC AAA TCT ACC CTG AAG CCG GAA GGC CAG CAG GCT CTG GAT CAG CTG TAC AGC CAG
F   N   K   S   T   L   K   P   E   G   Q   Q   A   L   D   Q   L   Y   S   Q
841/281                                          871/291
CTG AGC AAC CTG GAT CCG AAA GAC GGT TCC GTT GTC GTT CTG GGC TTC ACT GAC CGT ATC
  L   S   N   L   D   P   K   D   G   S   V   V   V   L   G   F   T   D   R   I
  901/301                                          931/311
GGT TCT GAC GCT TAC AAC CAG GGT CTG TCC GAG AAA CGT GCT CAG TCT GTT GTT GAT TAC
G   S   D   A   Y   N   Q   G   L   S   E   K   R   A   Q   S   V   V   D   Y
961/321                                          991/331
CTG ATC TCC AAA GGT ATT CCG TCT GAC AAA ATC TCC GCA CGT GGT ATG GGC GAA TCT AAC
  L   I   S   K   G   I   P   S   D   K   I   S   A   R   G   M   G   E   S   N
  1021/341                                         1051/351
CCG GTT ACC GGC AAC ACC TGT GAC AAC GTG AAA CCT CGC GCT GCC CTG ATC GAT TGC CTG
P   V   T   G   N   T   C   D   N   V   K   P   R   A   A   L   I   D   C   L
1081/361                                         1111/371
GCT CCG GAT CGT CGC GTA GAG ATC GAA GTT AAA GGC GTT AAA GAC GTG GTA ACT CAG CCG
  A   P   D   R   R   V   E   I   E   V   K   G   V   K   D   V   V   T   Q   P
  1141/381
CAG GCT TAA (SEQ ID NO:75 )
Q   A   *   (SEQ ID NO:76 )
```

OmpA Δ70-107::Cj1433 aa21-86

```
ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGCTCCGAAAGAT
AACACCTGGTACGCTGGTGCTAAACTGGGCTGGTCTCAGTACCATGACACCGGCTTCATTCACAATGATGGCCCG
ACTCATGAAAACCAACTGGGCGCAGGTGCTTTTGGTGGTTACCAGGTTAACCCGTATGTCGACATCAAAATCGAC
CTGAACAACACCAAAATCGACCTGAACAACACCAAAATCGACCTGAACAACACCAAAATCGACCTGAACAACACC
AAAATCGACCTGAACAACACCAAAATCGACCTGAACAACACCAAAATCGACCTGAACAACACCAAAATCGACCTG
AACAACACCAAAATCGACCTGAACAACACCAAAATCCTGCAGATCACTGACGATCTGGACGTTTATACCCGTCTG
GGTGGTATGGTATGGCGTGCAGACACCAAGTCTAACGTCCCTGGCGGCCCGTCTACTAAAGACCACGACACCGGC
GTTTCCCCGGTATTCGCGGGCGGTATCGAGTATGCTATCACCCCTGAAATCGCAACCCGTCTGGAATACCAGTGG
ACTAACAACATCGGTGATGCCAACACCATCGGCACCCGTCCGGACAACGGCCTGCTGAGCGTAGGTGTTTCCTAC
CGTTTCGGCCAGCAAGAAGCTGCTCCGGTAGTAGCTCCGGCACCAGCTCCGGCTCCGGAAGTACAGACCAAGCAC
TTCACTCTGAAGTCTGACGTACTGTTCAACTTCAACAAATCTACCCTGAAGCCGGAAGGCCAGCAGGCTCTGGAT
CAGCTGTACAGCCAGCTGAGCAACCTGGATCCGAAAGACGGTTCCGTTGTCGTTCTGGGCTTCACTGACCGTATC
GGTTCTGACGCTTACAACCAGGGTCTGTCCGAGAAACGTGCTCAGTCTGTTGTTGATTACCTGATCTCCAAAGGT
ATTCCGTCTGACAAAATCTCCGCACGTGGTATGGGCGAATCTAACCCGGTTACCGGCAACACCTGTGACAACGTG
AAACCTCGCGCTGCCCTGATCGATTGCCTGGCTCCGGATCGTCGCGTAGAGATCGAAGTTAAAGGCGTTAAAGAC
GTGGTAACTCAGCCGCAGGCTTAA (SEQ ID NO: 77)
```

[00380] **Figs. 8A-8B** depict the structural aspects of the modified *S.* Typhimurium OmpA protein with the insertion of the Cj1433c gene encoded sequences specifying the nine repeats to which the *C. jejuni* conserved N-glycan is attached as a consequence of the *C. jejuni pgl* operon and the activity of the *Salmonella* WaaL enzyme. The resulting construct is currently being introduced into a suicide vector that will be used to introduce the sequence encoding this Cj14433c-OmpA fusion into the χ12445 chromosome in place of the wild-type *ompA* gene. This strain will then be further modified to deliver a lysis plasmid encoding the *C. jeuni* antigens found in earlier studies to be maximally effective in reducing cecal titers of *C. jejuni* challenge strains. Experiments as described above by immunization of white leghorn and broiler chicks will be repeated.

Production of IROMPS and MnOMPs to enhance protective immunity against Salmonella serotypes and other enteric bacteria.

[00381] The ΔP_{fur}::TT *araC* P_{BAD} *fur* mutation was inserted into χ12341 to yield χ12396 (**Table 2**) and the ΔP_{mntR}::TT *araC* P_{BAD} *mntR* deletion-insertion mutation (**Table 3**) will also be inserted to lead to the up-regulation in vivo of all genes needed for iron and manganese uptake. Since immune responses to IROMPs are cross reactive in the *Enterobacteriaceae,* this strain, as LPS O-antigen synthesis ceases in vivo (due to the *pmi* mutation and absence of mannose and the *araC* P_{araBAD} regulated shutoff of the *waaL* gene) to better expose surface protein antigens, will induce enhanced cross-protective immunity to *Salmonella* serotypes and other enterics.

Example 4: Evaluation of RASV-Cj strains in white leghorn chickens

Evaluation of ability to induce cross-protective immunity to Salmonella serotypes, to prevent organ colonization, and to reduce cecal titers.

[00382] RASV-Cj will be evaluated delivering CjaA specified by the pG8R17 T2SS vector pG8R86 (**Fig. 8**) comparing χ12341 with the derivative of χ12396 described in Example 2. The protocol described by Hassan and Curtiss (113) will be followed. The CjaA antigen is selected for these comparative studies since in an unpublished study with one of the new RASV strains there was a 3- to 4-log reduction in cecal titers of the *C. jejuni* challenge strain. In addition, CjaA, which has been evaluated in some 23 prior studies, contains a sequence that should permit glycosylation with the *C. jejuni* N-glycan in the RASV-Cj strain. Day-of-hatch white leghorn chicks will be orally immunized and provided a second immunization at 10 days. At six weeks of age, ten immunized chicks and ten unimmunized chicks will each be challenged with 1 X 10^6 CFU of each of the eight serotypes of *Salmonella* listed in **Table 5** (Typhimurium, Enteritidis, Heidelberg, Montevideo, Hadar, Infantis, Newport and Kentucky). Four and eleven days after challenge, five birds in each group will be euthanized by CO_2 asphyxiation and titers of challenge and vaccine strains in liver, spleen, ovary (if present), bursa of Fabricius and cecum quantitated by direct plating on SS agar and by selenite-cysteine broth enrichment (113). The enrichment broth will detect bacterial titers as low as 5 per gram of tissue or contents. The biological containment features of the RASVs will preclude their detection.

Second stage studies.

[00383] These studies are repeated with a mixture of the two RASVs each delivering two or three validated protective *C. jejuni* antigens. The same immunization protocol described above will be used. This will serve as an independent repeat of the first experiment and establish the level of repeatability of the initial results. Titers of *S.* Typhimurium and *S.* Enteritidis challenge strains will also be evaluated 10, 20 and 30 days after challenge by immunizing with a combination of the two RASVs each delivering multiple *C. jejuni* protein antigens. Another small study will be directed at determining whether a single immunization is sufficient and determining how soon after primary immunization protective immunity to *Salmonella* serotypes is detectable in challenge studies.

Example 5: Evaluation of RASV-Cj strains in broiler chickens

[00384] RASV-Cj strains comprising lysis plasmids encoding each of the *C. jejuni* antigens listed in **Table 4** will be evaluated to determine the level of protection against *C. jejuni* colonization and persistence. Initial tests will evaluate whether χ12341 (**Figs. 4A** and **4B**) or χ12452 (**Fig. 5**) delivery of the CjaA antigen gives the best results. The best delivery strain will then be used in subsequent evaluations. Protection will be assessed by measuring CFU in cecal contents and the results correlated with antibody responses in both serum and feces. Plasmids encoding *C. jejuni* antigens resulting in the greatest levels of protection will be used to evaluate protection against heterologous challenge using well characterized *C. jejuni* strains, as well as to a cocktail of chicken isolates that are frequently isolated from poultry in the US (listed in **Table 5**).

[00385] Newly hatched day-old broiler chicks will be obtained from hatcheries, and prior to use, chicks will be confirmed negative for *Campylobacter* by culturing cloacal swabs on MH agar plates and by PCR

[00386] 6 groups of 3-day old broiler chickens (n=10/group) will be immunized with 1 X 10^9 CFU/chick orally with different RASV-Cj strains delivering *C. jejuni* antigens (**Table 4**). Control groups of 10 birds will be inoculated with RASV-Cj with an empty plasmid or be non-vaccinated. Chicks will be re-inoculated with RASVs 10 days after the first inoculation. Two weeks after the boost, chicks will be challenged with *C. jejuni* strain 81-176 orally (1 X 10^5 CFU/chick in 200 µl of PBS). Our previous work using 81-176 and a chicken *C. jejuni* cocktail indicated that this dose results in 100% colonization (186, 211). One week following challenge, chickens will be killed, cecal contents collected, homogenized in PBS, and plated on MH agar+CSS plates. Plates will be incubated at 42°C

microaerobically for 3 days and CFU/g of cecal contents determined to assess protection against *C. jejuni* challenge comparing titers from the vaccinated groups compared to the non-vaccinated control groups. It is expected that several RASV-Cj constructs will lead to over a 100-fold reduction in cecal *C. jejuni* colonization titers.

[00387] Blood, bile and fecal samples will be collected, IgY responses in serum and IgA responses in feces will be measured by indirect ELISA as previously described (212). Briefly, microtiter plates will be coated with purified recombinant *C. jejuni* proteins, washed, blocked with Sea Block blocking buffer (Pierce, Rockford, IL). Test samples (serum, bile or feces) will be added, the plates washed and anti-*C. jejuni* antibodies detected with biotinylated goat anti-chicken IgG or IgA antibody (Bethyl Laboratories, Montgomery, TX). Plates will be washed and streptavidin-horseradish peroxidase solution (Southern Biotech, Birmingham, AL) will be added. Color development will be detected using ABTS (Sigma, St. Louis, MO) containing 0.03% H_2O_2 in citrate buffer, pH 4.35. After color development, the reaction will be stopped with 1% SDS solution and OD_{405} measured using a microplate reader (Molecular Devices, Sunnyvale, CA).

[00388] Delivery of multiple *C. jejuni* antigens found to confer reduced challenge titers following immunization are tested. Plasmids encoding the multiple antigens will be constructed and introduced into the RASV-Cj strains. Studies will involve comparing one RASV versus a mixture of the two delivering the same or even different *C. jejuni* antigens. Immunizing with one versus two doses will be compared. If results with a single dose give satisfactory results, lower oral doses will be investigated. Ability of these RASVs to confer protection against *C. jejuni* cecal colonization will be assessed as described above. The protective response will also be correlated with antibody response in sera and feces as above. Control groups of chickens inoculated with a RASV carrying an empty plasmid and challenged and non-vaccinated unchallenged chickens will be used.

[00389] RASVs selected from the above studies are tested for whether they confer protection against challenge with heterologous *C. jejuni* strains. RASV-immunized chickens will be challenged with commonly used well-characterized *C. jejuni* strains (NC11168, 81116, RM221 and 81-176). In addition, chickens will be challenged with a cocktail of *C. jejuni* isolates of poultry origin (5 isolates that are frequently isolated from poultry). Chickens (n=10/group) will be vaccinated with selected RASVs (1×10^9 CFU/chicken orally) by the best means as established by the studies described above and challenged with 1×10^5 CFU/chick in 200 µl of PBS of *C. jejuni* strains. For the cocktail,

equal concentrations of each strain (1 X 10^5) will be mixed in PBS for challenge. Following challenge, protection against cecal colonization and antibody responses in serum and feces will be assessed as above. Groups of chickens inoculated with RASVs carrying an empty plasmid and challenged with *C. jejuni* as well as a group of non-vaccinated non-challenged chickens will be used as controls.

[00390] In regard to safety and utility, multiple mutations in all *S.* Typhimurium strains are included that preclude biofilm formation and inability to colonize gallstones in presence of bile. These same mutations decrease ability for persistence in the intestinal tracts of mice and chickens. *S.* Typhi strains with some of the same mutations in the proposed *S.* Typhimurium RASVs were unable to cause bacteremia in humans and were not shed in a viable cultivatable form in stools following oral administration of up to 10^{10} CFU (150). *S.* Typhimurium strains of the same genotype were safe in newborn (146, 147), pregnant, malnourished and immunocompromised mice. In addition, most of the strains undergo regulated delayed lysis which precludes persistence in vivo and viability if shed in feces (142). Also, infectivity and full virulence of these RASVs requires the presence of the sugars arabinose, mannose and rhamnose and thus inability if shed to infect or immunize animals or humans. New means to evaluate and demonstrate safety and efficacy of RASVs are continually devised, often with productive input from APHIS and FDA. Studies on survival of RASVs in water (with and without chlorination), as a consequence of desiccation, in sewage, in feces, in whole blood, in sera with and without complement inactivation, and in monocyte-derived macrophages have been performed. Such testing of *S.* Typhimurium RASVs and in chicken feces, body fluids and cells will ultimately be done.

[00391] While *Salmonella* is a category 2 pathogen with research conducted using BSL2 laboratory facilities and procedures and animal studies in ABSL2 facilities, the NIH RAC has reviewed the safety properties of our RASVs and is permitting conduct of all studies with immunization to be done under level 1 containment, in commercial agricultural settings and in outpatient human volunteers. This approval was contingent upon concurrence by the Institutional Biosafety Committee and the UF IBC has given such approval. However, challenge of immunized animals must still be conducted under the level of containment needed for that particular pathogen.

REFERENCES

1. **Altekruse SF, Stern NJ, Fields PI, Swerdlow DL.** 1999. *Campylobacter jejuni*--an emerging foodborne pathogen. Emerg Infect Dis **5:**28-35.
2. **DuPont HL.** 2007. The growing threat of foodborne bacterial enteropathogens of animal origin. Clin Infect Dis **45:**1353-1361.
3. **Lee MD, Newell DG.** 2006. *Campylobacter* in poultry: filling an ecological niche. Avian Dis **50:**1-9.
4. **Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, Van Immerseel F.** 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiol Rev **33:**718-738.
5. **Braden CR.** 2006. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis **43:**512-517.
6. **Bhavsar AP, Zhao X, Brown ED.** 2001. Development and characterization of a xylose-dependent system for expression of cloned genes in *Bacillus subtilis*: conditional complementation of a teichoic acid mutant. Appl Environ Microbiol **67:**403-410.
7. **Walder RY, Walder JA.** 1986. Oligodeoxynucleotide-directed mutagenesis using the yeast transformation system. Gene **42:**133-139.
8. **Bauer CE, Hesse SD, Waechter-Brulla DA, Lynn SP, Gumport RI, Gardner JF.** 1985. A genetic enrichment for mutations constructed by oligodeoxynucleotide-directed mutagenesis. Gene **37:**73-81.
9. **Craik CS.** 1985. Use Of Oligonucleotides For Site-specific Mutagenesis BIOTECHNIQUES **3:**12-19.
10. **Smith M, Gillam S.** 1981. Constructed Mutants Using Synthetic Oligodeoxyribonucleotides as Site-Specific Mutagens, p 1-32. *In* Setlow JK, Hollaender A (ed), Genetic Engineering: Principles and Methods Volume 3 doi:10.1007/978-1-4615-7075-2_1. Springer US, Boston, MA.
11. **Mark DF, Lin LS, Lu SY.** May 21, 1985 1985. Human recombinant interleukin-2 muteins.
12. **Mark DF, Lin LS, Lu SDY.** 1988. Structural genes, plasmids and transformed cells for producing cysteine depleted muteins of interferon-β patent US 4737462
13. **Nothaft H, Scott NE, Vinogradov E, Liu X, Hu R, Beadle B, Fodor C, Miller WG, Li J, Cordwell SJ, Szymanski CM.** 2012. Diversity in the protein N-glycosylation pathways within the *Campylobacter* genus. Mol Cell Proteomics **11:**1203-1219.
14. **Szymanski CM, Michael FS, Jarrell HC, Li J, Gilbert M, Larocque S, Vinogradov E, Brisson JR.** 2003. Detection of conserved N-linked glycans and phase-variable lipooligosaccharides and capsules from campylobacter cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. J Biol Chem **278:**24509-24520.
15. **Wacker M, Linton D, Hitchen PG, Nita-Lazar M, Haslam SM, North SJ, Panico M, Morris HR, Dell A, Wren BW, Aebi M.** 2002. N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*. Science **298:**1790-1793.
16. **Elhenawy W, Bording-Jorgensen M, Valguarnera E, Haurat MF, Wine E, Feldman MF.** 2016. LPS Remodeling Triggers Formation of Outer Membrane Vesicles in *Salmonella*. MBio **7:**e00940-00916.

17. **Man SM, Hopkins LJ, Nugent E, Cox S, Gluck IM, Tourlomousis P, Wright JA, Cicuta P, Monie TP, Bryant CE.** 2014. Inflammasome activation causes dual recruitment of NLRC4 and NLRP3 to the same macromolecular complex. Proc Natl Acad Sci U S A **111:**7403-7408.
18. **Collins LV, Attridge S, Hackett J.** 1991. Mutations at *rfc* or *pmi* attenuate *Salmonella typhimurium* virulence for mice. Infect Immun **59:**1079-1085.
19. **Curtiss R, III., Zhang X, Wanda SY, Kang HY, Konjufca V, Li Y, Gunn B, Wang S, Scarpellini G, Lee IS.** 2007. Induction of host immune responses using *Salmonella*-vectored vaccines, p 297-313. *In* Brogden KA, Minion FC, Cornick N, Stanton TB, Zhang Q, Nolan LK, Wannemuehler MJ (ed), Virulence mechanisms of bacterial pathogens, 4th ed. ASM Press, Washington D.C.
20. **Cascales E, Buchanan SK, Duche D, Kleanthous C, Lloubes R, Postle K, Riley M, Slatin S, Cavard D.** 2007. Colicin biology. Microbiol Mol Biol Rev **71:**158-229.
21. **Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ.** 2005. Disease risks from foods, England and Wales, 1996-2000. Emerg Infect Dis **11:**365-372.
22. **Allos BM, Moore MR, Griffin PM, Tauxe RV.** 2004. Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. Clin Infect Dis **38 Suppl 3:**S115-120.
23. **Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV.** 1999. Food-related illness and death in the United States. Emerg Infect Dis **5:**607-625.
24. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis **17:**7-15.
25. **Nachamkin I, Allos BM, Ho T.** 1998. *Campylobacter* species and Guillain-Barre syndrome. Clin Microbiol Rev **11:**555-567.
26. **Doorduyn Y, Van Den Brandhof WE, Van Duynhoven YT, Breukink BJ, Wagenaar JA, Van Pelt W.** 2010. Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. Epidemiol Infect **138:**1391-1404.
27. **Ge B, Girard W, Zhao S, Friedman S, Gaines SA, Meng J.** 2006. Genotyping of *Campylobacter spp.* from retail meats by pulsed-field gel electrophoresis and ribotyping. J Appl Microbiol **100:**175-184.
28. **Mullner P, Collins-Emerson JM, Midwinter AC, Carter P, Spencer SE, van der Logt P, Hathaway S, French NP.** 2010. Molecular epidemiology of *Campylobacter jejuni* in a geographically isolated country with a uniquely structured poultry industry. Appl Environ Microbiol **76:**2145-2154.
29. **Mullner P, Shadbolt T, Collins-Emerson JM, Midwinter AC, Spencer SE, Marshall J, Carter PE, Campbell DM, Wilson DJ, Hathaway S, Pirie R, French NP.** 2010. Molecular and spatial epidemiology of human campylobacteriosis: source association and genotype-related risk factors. Epidemiol Infect **138:**1372-1383.
30. **Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle PJ.** 2008. Tracing the source of campylobacteriosis. PLoS Genet **4:**e1000203.

31. **Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B, Tauxe RV, Emerging Infections Program FoodNet Working G.** 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin Infect Dis **38 Suppl 3:**S285-296.
32. **Friedman CR, Niemann J, Wegener HC, Tauxe RV.** 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p 121-138. *In* Nachamkin I, Blaser MJ (ed), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
33. **Guerin MT, Sir C, Sargeant JM, Waddell L, O'Connor AM, Wills RW, Bailey RH, Byrd JA.** 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: a systematic review. Poult Sci **89:**1070-1084.
34. **Strachan NJ, Gormley FJ, Rotariu O, Ogden ID, Miller G, Dunn GM, Sheppard SK, Dallas JF, Reid TM, Howie H, Maiden MC, Forbes KJ.** 2009. Attribution of *Campylobacter* infections in northeast Scotland to specific sources by use of multilocus sequence typing. J Infect Dis **199:**1205-1208.
35. **Corry JE, Atabay HI.** 2001. Poultry as a source of *Campylobacter* and related organisms. Symp Ser Soc Appl Microbiol:96S-114S.
36. **Sahin O, Kobalka P, Zhang Q.** 2003. Detection and survival of *Campylobacter* in chicken eggs. J Appl Microbiol **95:**1070-1079.
37. **Shane SM.** 1992. The significance of *Campylobacter jejuni* infection in poultry: a review. Avian Pathol **21:**189-213.
38. **Newell DG, Fearnley C.** 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl Environ Microbiol **69:**4343-4351.
39. **Zhang Q, P. P.** 2008. Mechanisms of antibiotic resistance in *Campylobacter.*, p 263–276. *In* Nachamkin I, Szymanski CM, Blaser MJ (ed), *Campylobacter* vol III. ASM Press; Washington DC, USA.
40. **Stern NJ, Fedorka-Cray P, Bailey JS, Cox NA, Craven SE, Hiett KL, Musgrove MT, Ladely S, Cosby D, Mead GC.** 2001. Distribution of *Campylobacter spp.* in selected U.S. poultry production and processing operations. J Food Prot **64:**1705-1710.
41. **Luangtongkum T, Morishita TY, Ison AJ, Huang S, McDermott PF, Zhang Q.** 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter spp.* in poultry. Appl Environ Microbiol **72:**3600-3607.
42. **Food Standards Agency UK.** 2008. A critical review of interventions and strategies (both biosecurity and non-biosecurity) to reduce *Campylobacter* on the poultry farm. B15025
43. **Gregory E, Barnhart H, Dreesen DW, Stern NJ, Corn JL.** 1997. Epidemiological study of *Campylobacter spp.* in broilers: source, time of colonization, and prevalence. Avian Dis **41:**890-898.
44. **Cardinale E, Tall F, Gueye EF, Cisse M, Salvat G.** 2004. Risk factors for *Campylobacter spp.* infection in Senegalese broiler-chicken flocks. Prev Vet Med **64:**15-25.
45. **Berndtson E, Danielsson-Tham ML, Engvall A.** 1996. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. Int J Food Microbiol **32:**35-47.

46. **Barrios PR, Reiersen J, Lowman R, Bisaillon JR, Michel P, Fridriksdottir V, Gunnarsson E, Stern N, Berke O, McEwen S, Martin W.** 2006. Risk factors for *Campylobacter spp.* colonization in broiler flocks in Iceland. Prev Vet Med **74:**264-278.
47. **Stern NJ, Pretanik S.** 2006. Counts of *Campylobacter spp.* on U.S. broiler carcasses. J Food Prot **69:**1034-1039.
48. **Wempe JM, Genigeorgis CA, Farver TB, Yusufu HI.** 1983. Prevalence of *Campylobacter jejuni* in two California chicken processing plants. Appl Environ Microbiol **45:**355-359.
49. **Richardson LJ, Cox NA, Bailey JS, Berrang ME, Cox JM, Buhr RJ, Fedorka-Cray PJ, Harrison MA.** 2009. Evaluation of TECRA broth, Bolton broth, and direct plating for recovery of *Campylobacter spp*, from broiler carcass rinsates from commercial processing plants. J Food Prot **72:**972-977.
50. **Logue CM, Sherwood JS, Elijah LM, Olah PA, Dockter MR.** 2003. The incidence of *Campylobacter spp.* on processed turkey from processing plants in the midwestern United States. J Appl Microbiol **95:**234-241.
51. **Hue O, Le Bouquin S, Laisney MJ, Allain V, Lalande F, Petetin I, Rouxel S, Quesne S, Gloaguen PY, Picherot M, Santolini J, Salvat G, Bougeard S, Chemaly M.** 2010. Prevalence of and risk factors for *Campylobacter spp.* contamination of broiler chicken carcasses at the slaughterhouse. Food Microbiol **27:**992-999.
52. **EFSA.** 2011. Annual report 2011.
53. **Berrang ME, Bailey JS, Altekruse SF, Patel B, Shaw WK, Jr., Meinersmann RJ, Fedorka-Cray PJ.** 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 U.S. processing plants. J Food Prot **70:**1556-1560.
54. **Nannapaneni R, Story R, Wiggins KC, Johnson MG.** 2005. Concurrent quantitation of total *campylobacter* and total ciprofloxacin-resistant *campylobacter* loads in rinses from retail raw chicken carcasses from 2001 to 2003 by direct plating at 42 degrees C. Appl Environ Microbiol **71:**4510-4515.
55. **Byrd JA, Sams AR, Hargis BM, Caldwell DJ.** 2011. Effect of selected modified atmosphere packaging on *Campylobacter* survival in raw poultry. Poult Sci **90:**1324-1328.
56. **Rosenquist H, Sommer HM, Nielsen NL, Christensen BB.** 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. Int J Food Microbiol **108:**226-232.
57. **Jeon B, Muraoka WT, Zhang Q.** 2010. Advances in *Campylobacter* biology and implications for biotechnological applications. Microb Biotechnol **3:**242-258.
58. **Hue O, Allain V, Laisney MJ, Le Bouquin S, Lalande F, Petetin I, Rouxel S, Quesne S, Gloaguen PY, Picherot M, Santolini J, Bougeard S, Salvat G, Chemaly M.** 2011. *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination. Food Microbiol **28:**862-868.
59. **Berrang ME, Smith DP, Windham WR, Feldner PW.** 2004. Effect of intestinal content contamination on broiler carcass *Campylobacter* counts. J Food Prot **67:**235-238.
60. **Allen VM, Bull SA, Corry JE, Domingue G, Jorgensen F, Frost JA, Whyte R, Gonzalez A, Elviss N, Humphrey TJ.** 2007. *Campylobacter spp.*

contamination of chicken carcasses during processing in relation to flock colonisation. Int J Food Microbiol **113:**54-61.

61. **Andrews-Polymenis HL, Baumler AJ, McCormick BA, Fang FC.** 2010. Taming the elephant: *Salmonella* biology, pathogenesis, and prevention. Infect Immun **78:**2356-2369.
62. **Hoffmann S, Batz MB, Morris JG, Jr.** 2012. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J Food Prot **75:**1292-1302.
63. **Schleifer JH, Juven BJ, Beard CW, Cox NA.** 1984. The susceptibility of chicks to *Salmonella montevideo* in artificially contaminated poultry feed. Avian Dis **28:**497-503.
64. **Williams JE, Benson ST.** 1978. Survival of *Salmonella typhimurium* in poultry feed and litter at three temperatures. Avian Dis **22:**742-747.
65. **CDC.** 1997. *Salmonella* serotype Montevideo infections associated with chicks--Idaho, Washington, and Oregon, spring 1995 and 1996. MMWR Morb Mortal Wkly Rep **46:**237-239.
66. **Batz MB, Doyle MP, Morris G, Jr., Painter J, Singh R, Tauxe RV, Taylor MR, Lo Fo Wong DM, Food Attribution Working G.** 2005. Attributing illness to food. Emerg Infect Dis **11:**993-999.
67. **Morris Jr JG, Hoffmann S, Batz B.** 2011. Ranking the Risks: The 10 Pathogen-Food Combinations With the Greatest Burden on Public Health.
68. **Batz MB, Hoffmann S, Morris Jr JG.** 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection **75:**1278-1291.
69. **CDC.** 2009. Multistate outbreaks of *Salmonella* infections associated with live poultry--United States, 2007. MMWR Morb Mortal Wkly Rep **58:**25-29.
70. **CDC.** 2007. Three outbreaks of salmonellosis associated with baby poultry from three hatcheries--United States, 2006. MMWR Morb Mortal Wkly Rep **56:**273-276.
71. **Altekruse SF, Bauer N, Chanlongbutra A, DeSagun R, Naugle A, Schlosser W, Umholtz R, White P.** 2006. *Salmonella enteritidis* in broiler chickens, United States, 2000-2005. Emerg Infect Dis **12:**1848-1852.
72. **Patrick ME, Adcock PM, Gomez TM, Altekruse SF, Holland BH, Tauxe RV, Swerdlow DL.** 2004. *Salmonella enteritidis* infections, United States, 1985-1999. Emerg Infect Dis **10:**1-7.
73. **Linam WM, Gerber MA.** 2007. Changing epidemiology and prevention of *Salmonella* infections. Pediatr Infect Dis J **26:**747-748.
74. **CDC.** 2014. Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2012. (Final Report). U.S. Department of Health and Human Services, CDC., Atlanta, Georgia.
75. **CDC.** 2016. National Enteric Disease Surveillance: *Salmonella* Annual Report, 2013. U.S. Department of Health and Human Services, CDC., Atlanta, Georgia.
76. **CDC.** 2012. Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2011 (Final Report). . U.S. Department of Health and Human Services, CDC., Atlanta, Georgia: .

77. **Roy P, Dhillon AS, Lauerman LH, Schaberg DM, Bandli D, Johnson S.** 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis **46:**17-24.
78. **Kuehn BM.** 2010. *Salmonella* cases traced to egg producers: findings trigger recall of more than 500 million eggs. JAMA **304:**1316.
79. **Hennessy TW, Cheng LH, Kassenborg H, Ahuja SD, Mohle-Boetani J, Marcus R, Shiferaw B, Angulo FJ, Emerging Infections Program FoodNet Working G.** 2004. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: a case-control study in FoodNet sites. Clin Infect Dis **38 Suppl 3:**S237-243.
80. **Elson R, Little CL, Mitchell RT.** 2005. *Salmonella* and raw shell eggs: results of a cross-sectional study of contamination rates and egg safety practices in the United Kingdom catering sector in 2003. J Food Prot **68:**256-264.
81. **Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, Feldman J, Coleman WW, Edmonson LM, MacDonald KL, Osterholm MT.** 1996. A national outbreak of *Salmonella enteritidis* infections from ice cream. The Investigation Team. N Engl J Med **334:**1281-1286.
82. **Han J, David DE, Deck J, Lynne AM, Kaldhone P, Nayak R, Stefanova R, Foley SL.** 2011. Comparison of *Salmonella enterica* serovar Heidelberg isolates from human patients with those from animal and food sources. J Clin Microbiol **49:**1130-1133.
83. **Le Hello S, Hendriksen RS, Doublet B, Fisher I, Nielsen EM, Whichard JM, Bouchrif B, Fashae K, Granier SA, Jourdan-Da Silva N, Cloeckaert A, Threlfall EJ, Angulo FJ, Aarestrup FM, Wain J, Weill FX.** 2011. International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. J Infect Dis **204:**675-684.
84. **Ladely SR, Meinersmann RJ, Ball TA, Fedorka-Cray PJ.** 2016. Antimicrobial Susceptibility and Plasmid Replicon Typing of *Salmonella enterica* Serovar Kentucky Isolates Recovered from Broilers. Foodborne Pathog Dis **13:**309-315.
85. **Jones DR, Guard J, Gast RK, Buhr RJ, Fedorka-Cray PJ, Abdo Z, Plumblee JR, Bourassa DV, Cox NA, Rigsby LL, Robison CI, Regmi P, Karcher DM.** 2016. Influence of commercial laying hen housing systems on the incidence and identification of *Salmonella* and *Campylobacter*. Poult Sci **95:**1116-1124.
86. **Bailar JC, 3rd, Travers K.** 2002. Review of assessments of the human health risk associated with the use of antimicrobial agents in agriculture. Clin Infect Dis **34 Suppl 3:**S135-143.
87. **Cohen ML, Tauxe RV.** 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science **234:**964-969.
88. **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N.** 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis **10:**597-602.

89. **Bebell LM, Muiru AN.** 2014. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? Glob Heart **9:**347-358.
90. **Founou LL, Founou RC, Essack SY.** 2016. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. Front Microbiol **7:**1881.
91. **Founou RC, Founou LL, Essack SY.** 2017. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One **12:**e0189621.
92. **Prestinaci F, Pezzotti P, Pantosti A.** 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health **109:**309-318.
93. **O'Brien TF.** 1997. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. Clin Infect Dis **24 Suppl 1:**S2-8.
94. **Centres for Disease Control and Prevention UDoHaHS.** 2013. Antibiotic resistance threats in the United States. https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed
95. **Organization WH.** 2001. WHO global strategy for containment of antimicrobial resistance. Accessed
96. **Organization WH.** 2012. The evolving threat of antimicrobial resistance. Options for action. Accessed
97. **Organization WH.** 2014. Antimicrobial resistance: global report on surveillance 2014. Accessed
98. **Casewell M, Friis C, Marco E, McMullin P, Phillips I.** 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother **52:**159-161.
99. **Lin J.** 2009. Novel approaches for *Campylobacter* control in poultry. Foodborne Pathog Dis **6:**755-765.
100. **Hermans D, Van Deun K, Messens W, Martel A, Van Immerseel F, Haesebrouck F, Rasschaert G, Heyndrickx M, Pasmans F.** 2011. *Campylobacter* control in poultry by current intervention measures ineffective: urgent need for intensified fundamental research. Vet Microbiol **152:**219-228.
101. **Doyle MP, Erickson MC.** 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult Sci **85:**960-973.
102. **Ridley AM, Morris VK, Cawthraw SA, Ellis-Iversen J, Harris JA, Kennedy EM, Newell DG, Allen VM.** 2011. Longitudinal molecular epidemiological study of thermophilic *campylobacters* on one conventional broiler chicken farm. Appl Environ Microbiol **77:**98-107.
103. **Ridley A, Morris V, Gittins J, Cawthraw S, Harris J, Edge S, Allen V.** 2011. Potential sources of *Campylobacter* infection on chicken farms: contamination and control of broiler-harvesting equipment, vehicles and personnel. J Appl Microbiol **111:**233-244.
104. **Arsenault J, Letellier A, Quessy S, Normand V, Boulianne M.** 2007. Prevalence and risk factors for *Salmonella spp.* and *Campylobacter spp.* caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. Prev Vet Med **81:**250-264.
105. **Nather G, Alter T, Martin A, Ellerbroek L.** 2009. Analysis of risk factors for *Campylobacter* species infection in broiler flocks. Poult Sci **88:**1299-1305.
106. **Hermans D, Van Deun K, Martel A, Van Immerseel F, Messens W, Heyndrickx M, Haesebrouck F, Pasmans F.** 2011. Colonization factors of *Campylobacter jejuni* in the chicken gut. Vet Res **42:**82.

107. **Layton SL, Morgan MJ, Cole K, Kwon YM, Donoghue DJ, Hargis BM, Pumford NR.** 2011. Evaluation of *Salmonella*-vectored *Campylobacter* peptide epitopes for reduction of *Campylobacter jejuni* in broiler chickens. Clin Vaccine Immunol **18:**449-454.

108. **Buckley AM, Wang J, Hudson DL, Grant AJ, Jones MA, Maskell DJ, Stevens MP.** 2010. Evaluation of live-attenuated *Salmonella* vaccines expressing *Campylobacter* antigens for control of *C. jejuni* in poultry. Vaccine **28:**1094-1105.

109. **Cawthraw S, Ayling R, Nuijten P, Wassenaar T, Newell DG.** 1994. Isotype, specificity, and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. Avian Dis **38:**341-349.

110. **Myszewski MA, Stern NJ.** 1990. Influence of *Campylobacter jejuni* cecal colonization on immunoglobulin response in chickens. Avian Dis **34:**588-594.

111. **Noor SM, Husband AJ, Widders PR.** 1995. In ovo oral vaccination with *Campylobacter jejuni* establishes early development of intestinal immunity in chickens. Br Poult Sci **36:**563-573.

112. **Sahin O, Zhang Q, Meitzler JC, Harr BS, Morishita TY, Mohan R.** 2001. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. Appl Environ Microbiol **67:**3951-3957.

113. **Hassan JO, Curtiss R, III.** 1994. Development and evaluation of an experimental vaccination program using a live avirulent *Salmonella typhimurium* strain to protect immunized chickens against challenge with homologous and heterologous *Salmonella* serotypes. Infect Immun **62:**5519-5527.

114. **Dorea FC, Cole DJ, Hofacre C, Zamperini K, Mathis D, Doyle MP, Lee MD, Maurer JJ.** 2010. Effect of *Salmonella* vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. Appl Environ Microbiol **76:**7820-7825.

115. **Gantois I, Ducatelle R, Timbermont L, Boyen F, Bohez L, Haesebrouck F, Pasmans F, van Immerseel F.** 2006. Oral immunisation of laying hens with the live vaccine strains of TAD *Salmonella* vac® E and TAD *Salmonella* vac® T reduces internal egg contamination with *Salmonella* Enteritidis. Vaccine **24:**6250-6255.

116. **Cooper GL, Venables LM, Woodward MJ, Hormaeche CE.** 1994. Vaccination of chickens with strain CVL30, a genetically defined *Salmonella enteritidis aroA* live oral vaccine candidate. Infect Immun **62:**4747-4754.

117. **Cerquetti MC, Gherardi MM.** 2000. Vaccination of chickens with a temperature-sensitive mutant of *Salmonella enteritidis*. Vaccine **18:**1140-1145.

118. **Springer S, Lindner T, Ahrens M, Woitow G, Prandini F, Selbitz HJ.** 2011. Duration of immunity induced in chickens by an attenuated live *Salmonella enteritidis* vaccine and an inactivated *Salmonella enteritidis/typhimurium* vaccine. Berl Munch Tierarztl Wochenschr **124:**89-93.

119. **Methner U, Barrow PA, Berndt A, Rychlik I.** 2011. *Salmonella* Enteritidis with double deletion in *phoPfliC*--a potential live *Salmonella* vaccine candidate with novel characteristics for use in chickens. Vaccine **29:**3248-3253.

120. **Nandre RM, Matsuda K, Chaudhari AA, Kim B, Lee JH.** 2012. A genetically engineered derivative of *Salmonella* Enteritidis as a novel live vaccine candidate for salmonellosis in chickens. Res Vet Sci **93:**596-603.
121. **Matulova M, Havlickova H, Sisak F, Rychlik I.** 2012. Vaccination of chickens with *Salmonella* Pathogenicity Island (SPI) 1 and SPI2 defective mutants of *Salmonella enterica* serovar Enteritidis. Vaccine **30:**2090-2097.
122. **Tan S, Gyles CL, Wilkie BN.** 1997. Evaluation of an *aroA* mutant *Salmonella typhimurium* vaccine in chickens using modified semisolid Rappaport Vassiliadis medium to monitor faecal shedding. Vet Microbiol **54:**247-254.
123. **Barrow PA.** 2007. *Salmonella* infections: immune and non-immune protection with vaccines. Avian Pathol **36:**1-13.
124. **Gast RK.** 2007. Serotype-specific and serotype-independent strategies for preharvest control of food-borne *Salmonella* in poultry. Avian Dis **51:**817-828.
125. **Desin TS, Koster W, Potter AA.** 2013. *Salmonella* vaccines in poultry: past, present and future. Expert Rev Vaccines **12:**87-96.
126. **Kaniuk NA, Monteiro MA, Parker CT, Whitfield C.** 2002. Molecular diversity of the genetic loci responsible for lipopolysaccharide core oligosaccharide assembly within the genus *Salmonella.* Mol Microbiol **46:**1305-1318.
127. **Malik M, Butchaiah G, Bansal MP, Siddiqui MZ, Bakshi CS, Singh RK.** 2002. Antigenic relationships within the genus *Salmonella* as revealed by anti-*Salmonella enteritidis* monoclonal antibodies. Vet Res Commun **26:**179-188.
128. **Earhart CF.** 1996. Uptake and metabolism of iron and molybdenum., p 1075-1090. *In* Neidhardt FC, Curtiss III R, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (ed), *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd ed, vol 1. ASM Press, Washington, D.C.
129. **Curtiss R, III., Wanda SY, Gunn BM, Zhang X, Tinge SA, Ananthnarayan V, Mo H, Wang S, Kong W.** 2009. *Salmonella enterica* serovar Typhimurium strains with regulated delayed attenuation in vivo. Infect Immun **77:**1071-1082.
130. **Bolin CA, Jensen AE.** 1987. Passive immunization with antibodies against iron-regulated outer membrane proteins protects turkeys from *Escherichia coli* septicemia. Infect Immun **55:**1239-1242.
131. **Lin J, Hogan JS, Smith KL.** 1999. Antigenic homology of the inducible ferric citrate receptor (FecA) of coliform bacteria isolated from herds with naturally occurring bovine intramammary infections. Clin Diagn Lab Immunol **6:**966-969.
132. **Wilmes-Riesenberg MR, Bearson B, Foster JW, Curtiss III R.** 1996. Role of the acid tolerance response in virulence of *Salmonella typhimurium*. Infect Immun **64:**1085-1092.
133. **Łaniewski P, Mitra A, Karaca K, Khan A, Prasad R, Curtiss R, III, Roland KL.** 2014. Evaluation of protective efficacy of live attenuated *Salmonella enterica* serovar Gallinarum vaccine strains against fowl typhoid in chickens. Clin Vaccine Immunol **21:**1267-1276.
134. **Li Y, Wang S, Scarpellini G, Gunn B, Xin W, Wanda SY, Roland KL, Curtiss R, III.** 2009. Evaluation of new generation *Salmonella enterica* serovar Typhimurium vaccines with regulated delayed attenuation to induce immune responses against PspA. Proc Natl Acad Sci U S A **106:**593-598.

135. **Shi H, Santander J, Brenneman KE, Wanda SY, Wang S, Senechal P, Sun W, Roland KL, Curtiss R, III.** 2010. Live recombinant *Salmonella* Typhi vaccines constructed to investigate the role of *rpoS* in eliciting immunity to a heterologous antigen. PLoS One **5:**e11142.
136. **Shi H, Wang S, Curtiss R, III.** 2013. Evaluation of regulated delayed attenuation strategies for *Salmonella enterica* serovar Typhi vaccine vectors in neonatal and infant mice. Clin Vaccine Immunol **20:**931-944.
137. **Wang S, Li Y, Scarpellini G, Kong W, Shi H, Baek CH, Gunn B, Wanda SY, Roland KL, Zhang X, Senechal-Willis P, Curtiss R, III.** 2010. *Salmonella* vaccine vectors displaying delayed antigen synthesis in vivo to enhance immunogenicity. Infect Immun **78:**3969-3980.
138. **Wang S, Li Y, Shi H, Sun W, Roland KL, Curtiss R, III.** 2011. Comparison of a regulated delayed antigen synthesis system with in vivo-inducible promoters for antigen delivery by live attenuated *Salmonella* vaccines. Infect Immun **79:**937-949.
139. **Xin W, Wanda SY, Li Y, Wang S, Mo H, Curtiss R, III.** 2008. Analysis of type II secretion of recombinant pneumococcal PspA and PspC in a *Salmonella enterica* serovar Typhimurium vaccine with regulated delayed antigen synthesis. Infect Immun **76:**3241-3254.
140. **Juárez-Rodríguez MD, Yang J, Kader R, Alamuri P, Curtiss R, III, Clark-Curtiss JE.** 2012. Live attenuated *Salmonella* vaccines displaying regulated delayed lysis and delayed antigen synthesis to confer protection against *Mycobacterium tuberculosis*. Infect Immun **80:**815-831.
141. **Kong W, Brovold M, Koeneman BA, Clark-Curtiss J, Curtiss R, III.** 2012. Turning self-destructing *Salmonella* into a universal DNA vaccine delivery platform. Proc Natl Acad Sci U S A **109:**19414-19419.
142. **Kong W, Wanda SY, Zhang X, Bollen W, Tinge SA, Roland KL, Curtiss R, III.** 2008. Regulated programmed lysis of recombinant *Salmonella* in host tissues to release protective antigens and confer biological containment. Proc Natl Acad Sci U S A **105:**9361-9366.
143. **Germanier R, Furer E.** 1971. Immunity in experimental salmonellosis. II. Basis for the avirulence and protective capacity of *gal* E mutants of *Salmonella typhimurium*. Infect Immun **4:**663-673.
144. **Kong Q, Liu Q, Roland KL, Curtiss R, III.** 2009. Regulated delayed expression of *rfaH* in an attenuated *Salmonella enterica* serovar Typhimurium vaccine enhances immunogenicity of outer membrane proteins and a heterologous antigen. Infect Immun **77:**5572-5582.
145. **Li Y, Wang S, Xin W, Scarpellini G, Shi Z, Gunn B, Roland KL, Curtiss R, III.** 2008. A *sopB* deletion mutation enhances the immunogenicity and protective efficacy of a heterologous antigen delivered by live attenuated *Salmonella enterica* vaccines. Infect Immun **76:**5238-5246.
146. **Gunn BM, Wanda SY, Burshell D, Wang C, Curtiss R, III.** 2010. Construction of recombinant attenuated *Salmonella enterica* serovar Typhimurium vaccine vector strains for safety in newborn and infant mice. Clin Vaccine Immunol **17:**354-362.
147. **Shi H, Wang S, Roland KL, Gunn BM, Curtiss R, III.** 2010. Immunogenicity of a live recombinant *Salmonella enterica* serovar Typhimurium vaccine expressing *pspA* in neonates and infant mice born from naive and immunized mothers. Clin Vaccine Immunol **17:**363-371.

148. **Hassan JO, Curtiss R, III.** 1996. Effect of vaccination of hens with an avirulent strain of *Salmonella typhimurium* on immunity of progeny challenged with wild-type *Salmonella* strains. Infection and Immunity **64:**938-944.
149. **Juárez-Rodríguez MD, Arteaga-Cortés LT, Kader R, Curtiss R, III, Clark-Curtiss JE.** 2012. Live attenuated *Salmonella* vaccines against *Mycobacterium tuberculosis* with antigen delivery via the type III secretion system. Infect Immun **80:**798-814.
150. **Frey SE, Lottenbach KR, Hill H, Blevins TP, Yu Y, Zhang Y, Brenneman KE, Kelly-Aehle SM, McDonald C, Jansen A, Curtiss R, III.** 2013. A Phase I, dose-escalation trial in adults of three recombinant attenuated *Salmonella* Typhi vaccine vectors producing *Streptococcus pneumoniae* surface protein antigen PspA. Vaccine **31:**4874-4880.
151. **Konjufca V, Jenkins M, Wang S, Juárez-Rodríguez MD, Curtiss R, III.** 2008. Immunogenicity of recombinant attenuated *Salmonella enterica* serovar Typhimurium vaccine strains carrying a gene that encodes *Eimeria tenella* antigen SO7. Infect Immun **76:**5745-5753.
152. **Konjufca V, Wanda SY, Jenkins MC, Curtiss R, III.** 2006. A recombinant attenuated *Salmonella enterica* serovar Typhimurium vaccine encoding *Eimeria acervulina* antigen offers protection against *E. acervulina* challenge. Infect Immun **74:**6785-6796.
153. **Zekarias B, Mo H, Curtiss R, III.** 2008. Recombinant attenuated *Salmonella enterica* serovar typhimurium expressing the carboxy-terminal domain of alpha toxin from *Clostridium perfringens* induces protective responses against necrotic enteritis in chickens. Clin Vaccine Immunol **15:**805-816.
154. **Jiang Y, Mo H, Willingham C, Wang S, Park JY, Kong W, Roland KL, Curtiss R, III.** 2015. Protection Against Necrotic Enteritis in Broiler Chickens by Regulated Delayed Lysis *Salmonella* Vaccines. Avian Dis **59:**475-485.
155. **Ashraf S, Kong W, Wang S, Yang J, Curtiss R, III.** 2011. Protective cellular responses elicited by vaccination with influenza nucleoprotein delivered by a live recombinant attenuated *Salmonella* vaccine. Vaccine **29:**3990-4002.
156. **Ameiss K, Ashraf S, Kong W, Pekosz A, Wu WH, Milich D, Billaud JN, Curtiss R, III.** 2010. Delivery of woodchuck hepatitis virus-like particle presented influenza M2e by recombinant attenuated *Salmonella* displaying a delayed lysis phenotype. Vaccine **28:**6704-6713.
157. **Nakayama K, Kelly SM, Curtiss R, III** 1988. Construction of an Asd^+ expression-cloning Vector: stable maintenance and high level expression of cloned genes in a *Salmonella* vaccine strain. Nat Biotech **6:**693-697.
158. **Branger CG, Sun W, Torres-Escobar A, Perry R, Roland KL, Fetherston J, Curtiss R, III.** 2010. Evaluation of Psn, HmuR and a modified LcrV protein delivered to mice by live attenuated *Salmonella* as a vaccine against bubonic and pneumonic *Yersinia pestis* challenge. Vaccine **29:**274-282.
159. **Kang HY, Srinivasan J, Curtiss R, III.** 2002. Immune responses to recombinant pneumococcal PspA antigen delivered by live attenuated *Salmonella enterica* serovar Typhimurium vaccine. Infect Immun **70:**1739-1749.
160. **Muralinath M, Kuehn MJ, Roland KL, Curtiss R, III.** 2011. Immunization with *Salmonella enterica* serovar Typhimurium-derived outer membrane vesicles delivering the pneumococcal protein PspA confers protection against challenge with *Streptococcus pneumoniae*. Infect Immun **79:**887-894.

161. **Kaniuk NA, Vinogradov E, Whitfield C.** 2004. Investigation of the structural requirements in the lipopolysaccharide core acceptor for ligation of O antigens in the genus *Salmonella*: WaaL "ligase" is not the sole determinant of acceptor specificity. J Biol Chem **279:**36470-36480.
162. **Curtiss R, III, Wanda SY, Gunn BM, Zhang X, Tinge SA, Ananthnarayan V, Mo H, Wang S, Kong W.** 2009. *Salmonella enterica* serovar Typhimurium strains with regulated delayed attenuation in vivo. Infect Immun **77:**1071-1082.
163. **Brosius J, Erfle M, Storella J.** 1985. Spacing of the -10 and -35 regions in the *tac* promoter. Effect on its in vivo activity. J Biol Chem **260:**3539-3541.
164. **Pizarro-Cerda J, Tedin K.** 2004. The bacterial signal molecule, ppGpp, regulates *Salmonella* virulence gene expression. Mol Microbiol **52:**1827-1844.
165. **Vander Byl C, Kropinski AM.** 2000. Sequence of the genome of *Salmonella* bacteriophage P22. J Bacteriol **182:**6472-6481.
166. **Brown ED, Vivas EI, Walsh CT, Kolter R.** 1995. MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in *Escherichia coli*. J Bacteriol **177:**4194-4197.
167. **Black S, Wright NG.** 1955. Aspartic β-semialdehyde dehydrogenase and aspartic β-semialdehyde. J Biol Chem **213:**39-50.
168. **Stevenson G, Andrianopoulos K, Hobbs M, Reeves PR.** 1996. Organization of the *Escherichia coli* K-12 gene cluster responsible for production of the extracellular polysaccharide colanic acid. J Bacteriol **178:**4885-4893.
169. **Whitfield C.** 2006. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. Annu Rev Biochem **75:**39-68.
170. **Beuzón CR, Méresse S, Unsworth KE, Ruiz-Albert J, Garvis S, Waterman SR, Ryder TA, Boucrot E, Holden DW.** 2000. *Salmonella* maintains the integrity of its intracellular vacuole through the action of SifA. EMBO J **19:**3235-3249.
171. **Cohen A, Laban A.** 1983. Plasmidic recombination in *Escherichia coli* K-12: the role of *recF* gene function. Mol Gen Genet **189:**471-474.
172. **Kolodner R, Fishel RA, Howard M.** 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. J Bacteriol **163:**1060-1066.
173. **Zhang X, Wanda SY, Brenneman K, Kong W, Roland K, Curtiss R, III.** 2011. Improving *Salmonella* vector with *rec* mutation to stabilize the DNA cargoes. BMC Microbiol **11:**31.
174. **Link C, Ebensen T, Standner L, Dejosez M, Reinhard E, Rharbaoui F, Guzman CA.** 2006. An SopB-mediated immune escape mechanism of *Salmonella enterica* can be subverted to optimize the performance of live attenuated vaccine carrier strains. Microbes Infect **8:**2262-2269.
175. **Norris FA, Wilson MP, Wallis TS, Galyov EE, Majerus PW.** 1998. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. Proc Natl Acad Sci U S A **95:**14057-14059.
176. **Reis BP, Zhang S, Tsolis RM, Baumler AJ, Adams LG, Santos RL.** 2003. The attenuated *sopB* mutant of *Salmonella enterica* serovar Typhimurium has the same tissue distribution and host chemokine response as the wild type in bovine Peyer's patches. Vet Microbiol **97:**269-277.

177. **Singh SP, Williams YU, Miller S, Nikaido H.** 2003. The C-terminal domain of *Salmonella enterica* serovar typhimurium OmpA is an immunodominant antigen in mice but appears to be only partially exposed on the bacterial cell surface. Infect Immun **71:**3937-3946.
178. **Chart H, Rowe B.** 1991. Antibodies to lipopolysaccharide and outer membrane proteins of *Salmonella enteritidis* PT4 are not involved in protection from experimental infection. FEMS Microbiol Lett **68:**345-350.
179. **Curtiss R, III., S. B. Porter, M. Munson, S. A. Tinge, J. O. Hassan, C. Gentry-Weeks, Kelly. SM.** 1991. Nonrecombinant and recombinant avirulent *Salmonella* live vaccines for poultry, p 169-198. *In* L. C. Blankenship, J.H.S. Bailey , N.A. Cox , Stern NJ, Meinersmann RJ (ed), Colonization control of human bacterial enteropathogens in poultry. Academic Press New York
180. **Poppe C, Gyles CL.** 1987. Relation of plasmids to virulence and other properties of salmonellae from avian sources. Avian Dis **31:**844-854.
181. **Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ.** 1988. Experimental *Campylobacter-Jejuni* Infection in Humans. Journal of Infectious Diseases **157:**472-479.
182. **Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S, Barrell BG.** 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature **403:**665-668.
183. **Pearson BM, Gaskin DJ, Segers RP, Wells JM, Nuijten PJ, van Vliet AH.** 2007. The complete genome sequence of *Campylobacter jejuni* strain 81116 (NCTC11828). J Bacteriol **189:**8402-8403.
184. **Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, Brinkac LM, DeBoy RT, Parker CT, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Sullivan SA, Shetty JU, Ayodeji MA, Shvartsbeyn A, Schatz MC, Badger JH, Fraser CM, Nelson KE.** 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *campylobacter* species. PLoS Biol **3:**e15.
185. **Parker CT, Quinones B, Miller WG, Horn ST, Mandrell RE.** 2006. Comparative genomic analysis of *Campylobacter jejuni* strains reveals diversity due to genomic elements similar to those present in *C. jejuni* strain RM1221. J Clin Microbiol **44:**4125-4135.
186. **Issmat I. Kassem, Gbenga K, Kumar A, Chandrashekhar K, Pina-Mimbela R, Rajashekara G.** 2014. An evaluation of the impact of litter chemical amendments on reducing *Campylobacter jejuni* in broilers, abstr Conference of research workers in animal disease (CRWAD), Chicago, IL., December 6-8.
187. **Zhang X, Kelly SM, Bollen WS, Curtiss R, III.** 1997. Characterization and immunogenicity of *Salmonella typhimurium* SL1344 and UK-1 Δ*crp* and Δ*cdt* deletion mutants. Infect Immun **65:**5381-5387.
188. **Kong Q, Liu Q, Jansen A, Curtiss R, III.** 2010. Regulated delayed expression of *rfc* enhances the immunogenicity and protective efficacy of a heterologous antigen delivered by live attenuated *Salmonella enterica* vaccines. Vaccine **28:**6094-6103.

189. **Bertani G.** 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J Bacteriol **62:**293-300.
190. **Pardee AB, Jacob F, Monod J.** 1959. The genetic control and cytoplasmic expression of "Inducibility" in the synthesis of β-galactosidase by *E. coli*. Journal of Molecular Biology **1:**165-178.
191. **Sambrook J, Russell DW.** 2001. Molecular cloning : a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
192. **Ehretsmann CP, Carpousis AJ, Krisch HM.** 1992. Specificity of *Escherichia coli* endoribonuclease RNase E: in vivo and in vitro analysis of mutants in a bacteriophage T4 mRNA processing site. Genes Dev **6:**149-159.
193. **McDowall KJ, Kaberdin VR, Wu SW, Cohen SN, Lin-Chao S.** 1995. Site-specific RNase E cleavage of oligonucleotides and inhibition by stem-loops. Nature **374:**287-290.
194. **Lin-Chao S, Wong TT, McDowall KJ, Cohen SN.** 1994. Effects of nucleotide sequence on the specificity of rne-dependent and RNase E-mediated cleavages of RNA I encoded by the pBR322 plasmid. J Biol Chem **269:**10797-10803.
195. **Edwards RA, Keller LH, Schifferli DM.** 1998. Improved allelic exchange vectors and their use to analyze 987P fimbria gene expression. Gene **207:**149-157.
196. **Kaniga K, Compton MS, Curtiss R, III., Sundaram P.** 1998. Molecular and functional characterization of *Salmonella enterica* serovar Typhimurium *poxA* gene: effect on attenuation of virulence and protection. Infect Immun **66:**5599-5606.
197. **Maloy SR, and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J Bacteriol **145:**1110-1112.
198. **Miller VL, and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J Bacteriol **170:**2575-2583.
199. **Ried J, and A. Collmer.** 1987. An *nptI-sacB-sacR* cartridge for constructing directed unmarked mutations in gram-negative bacteria by marker exchange-eviction mutagenesis. Gene **57:**239-246.
200. **Roland K, Curtiss R, III., Sizemore D.** 1999. Construction and evaluation of a Δ*cya* Δ*crp Salmonella typhimurium* strain expressing avian pathogenic *Escherichia coli* O78 LPS as a vaccine to prevent airsacculitis in chickens. Avian Dis **43:**429-441.
201. **Schmieger H.** 1972. Phage P22-mutants with increased or decreased transduction abilities. Mol Gen Genet **119:**75-88.
202. **Schmieger H, Backhaus H.** 1976. Altered cotransduction frequencies exhibited by HT-mutants of *Salmonella*-phage P22. Mol Gen Genet **143:**307-309.
203. **Quandt J, Hynes MF.** 1993. Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. Gene **127:**15-21.
204. **Hitchcock PJ, Brown TM.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J Bacteriol **154:**269-277.

205. **Kang HY, Dozois CM, Tinge SA, Lee TH, Curtiss R, III.** 2002. Transduction-mediated transfer of unmarked deletion and point mutations through use of counterselectable suicide vectors. J Bacteriol **184:**307-312.
206. **Young NM, Brisson JR, Kelly J, Watson DC, Tessier L, Lanthier PH, Jarrell HC, Cadotte N, St Michael F, Aberg E, Szymanski CM.** 2002. Structure of the N-linked glycan present on multiple glycoproteins in the Gram-negative bacterium, *Campylobacter jejuni*. J Biol Chem **277:**42530-42539.
207. **Hobbs M, Reeves PR.** 1994. The JUMPstart sequence: a 39 bp element common to several polysaccharide gene clusters. Mol Microbiol **12:**855-856.
208. **Nieto JM, Bailey MJ, Hughes C, Koronakis V.** 1996. Suppression of transcription polarity in the *Escherichia coli* haemolysin operon by a short upstream element shared by polysaccharide and DNA transfer determinants. Mol Microbiol **19:**705-713.
209. **Nothaft H, Davis B, Lock YY, Perez-Munoz ME, Vinogradov E, Walter J, Coros C, Szymanski CM.** 2016. Engineering the *Campylobacter jejuni* N-glycan to create an effective chicken vaccine. Sci Rep **6:**26511.
210. **Price NL, Goyette-Desjardins G, Nothaft H, Valguarnera E, Szymanski CM, Segura M, Feldman MF.** 2016. Glycoengineered Outer Membrane Vesicles: A Novel Platform for Bacterial Vaccines. Sci Rep **6:**24931.
211. **Gangaiah D, Liu Z, Arcos J, Kassem, II, Sanad Y, Torrelles JB, Rajashekara G.** 2010. Polyphosphate kinase 2: a novel determinant of stress responses and pathogenesis in *Campylobacter jejuni*. PLoS One **5:**e12142.
212. **Annamalai T, Pina-Mimbela R, Kumar A, Binjawadagi B, Liu Z, Renukaradhya GJ, Rajashekara G.** 2013. Evaluation of nanoparticle-encapsulated outer membrane proteins for the control of *Campylobacter jejuni* colonization in chickens. Poult Sci **92:**2201-2211.
213. **Reusch RN.** 2013. The role of short-chain conjugated poly-(R)-3-hydroxybutyrate (cPHB) in protein folding. Int J Mol Sci **14:**10727-10748.
214. **Pautsch A, Schulz GE.** 1998. Structure of the outer membrane protein A transmembrane domain. Nat Struct Biol **5:**1013-1017.
215. **Vogel H, Jahnig F.** 1986. Models for the structure of outer-membrane proteins of Escherichia coli derived from raman spectroscopy and prediction methods. J Mol Biol **190:**191-199.

CLAIMS

What is claimed is:

1. A recombinant derivative of a pathogenic bacterium comprising:
   a. a regulated-delayed attenuation;
   b. a regulated-delayed expression of an antigen of interest;
   c. a regulated-delayed lysis *in vivo* phenotype; and
   d. synthesizing and delivering one or more protein antigens of *Campylobacter jejuni.*

2. The bacterium of claim 1, wherein the bacterium further comprises mutations that cause display of the universal LPS core polysaccharide *in vivo.*

3. A recombinant derivative of a pathogenic bacterium comprising:
   a. a regulated-delayed attenuation;
   b. a regulated-delayed expression of an antigen of interest;
   c. a regulated-delayed lysis *in vivo* phenotype; and
   d. synthesizing and delivering one or more protein antigens that display the *Campylobacter jejuni* N-glycan.

4. A recombinant derivative of a pathogenic bacterium comprising:
   a. a regulated-delayed attenuation;
   b. a regulated-delayed expression of an antigen of interest;
   c. a regulated-delayed lysis *in vivo* phenotype; and
   d. synthesizing and delivering one or more protein antigens of *Campylobacter jejuni* and also displays the *Campylobacter jejuni* N-glycan.

5. The bacterium of any one of claims 1-4, wherein the bacterium comprises one or more *Campylobacter pgl* operon genes.

6. The bacterium of claim 5, wherein the one or more *Campylobacter pgl* operon genes are codon-optimized for expression in the bacterium.

7. The bacterium of any one of claims 1 to 6, wherein the bacterium is a Gram-negative bacterium.

8. The bacterium of any one of claims 1 to 7, wherein the bacterium belongs to the family *Enterobacteriaceae*.

9. The bacterium of any one of claims 1-8, wherein the antigen of interest is a *Campylobacter* antigen.

10. The bacterium of claim 9, wherein the antigen of interest is selected from the group consisting of Peb1, CjaA, Dps, TlyA, Omp18, Cj0998c, Cj0034c, Cj0168c, Cj0248, Peb3, CmeC, Cj0404, Cj0420, Cj0427, Cj0428, PorA, FlaA, CadF, and Cj1656c.

11. The bacterium of claim 3 or claim 4, wherein the *C. jejuni* N-glycan is attached to the antigen of interest.

12. The bacterium of claim 11, wherein the bacterium comprises insertion of all or part of the *cj1433c* gene into a deletion of the *ompA* gene to generate a fusion of the Cj1433c protein.

13. The bacterium of any one of claims 1 to 12, wherein the regulated-delayed attenuation is conferred by the gene *fur* or *mntR*.

14. The bacterium of any one of claims 1-13, wherein the regulated-delayed lysis *in vivo* phenotype is conferred by ΔP_{murA}::TT *araC* P_{BAD} *murA*, Δ*asdA*::TT *araC* P_{BAD} *c2*, or Δ(*wza-wcaM*) deletions or deletion/insertion mutations.

15. The bacterium of any one of claims 1-14, wherein the bacterium further comprises a mutation in the *sifA* gene.

16. The bacterium of any one of claims 1-15, wherein the bacterium further comprises a mutation in the *relA* gene.

17. A pharmaceutical composition comprising the recombinant bacterium of any one of claims 1-16, and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim 17, further comprising a second recombinant derivative of a pathogenic bacterium, wherein said bacterium comprises a nucleic acid encoding a second antigen of interest.

19. The pharmaceutical composition of claim 18, wherein the second antigen of interest is a *Salmonella* antigen.

20. A method for inducing protective immunity in an avian, the method comprising administering to the avian an effective amount of a pharmaceutical composition of any one of claims 17-19.

Fig. 1A

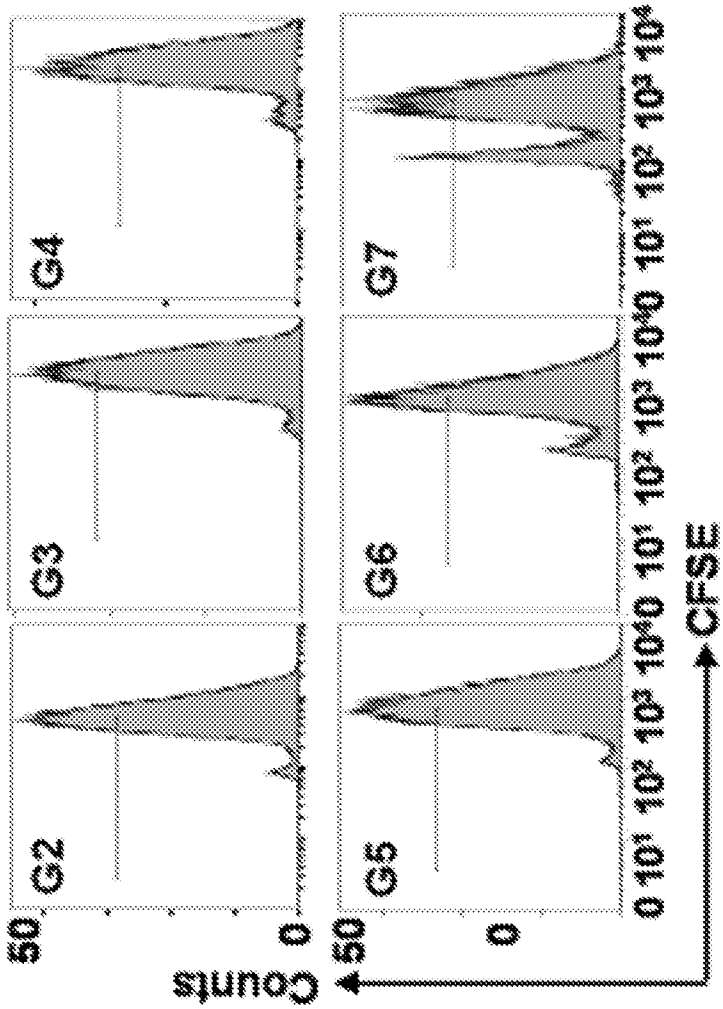
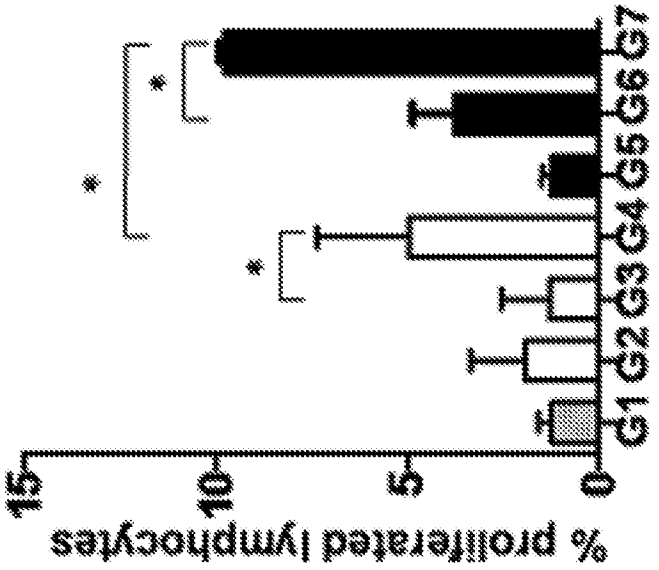


Fig. 1B



G1- BSG
G2-χ11840(pYA3342) non-lysis Δ*sifA* strain
G3-χ11442(pYA5301) non-lysis strain + SO7
G4-χ11840(pYA5301) non-lysis Δ*sifA* strain + SO7
G5-χ11791(pYA3681) lysis Δ*sifA* strain
G6-χ11730(pYA5293) lysis strain + SO7
G7-χ11791(pYA5293)) lysis Δ*sifA* strain + SO7

Fig. 1C

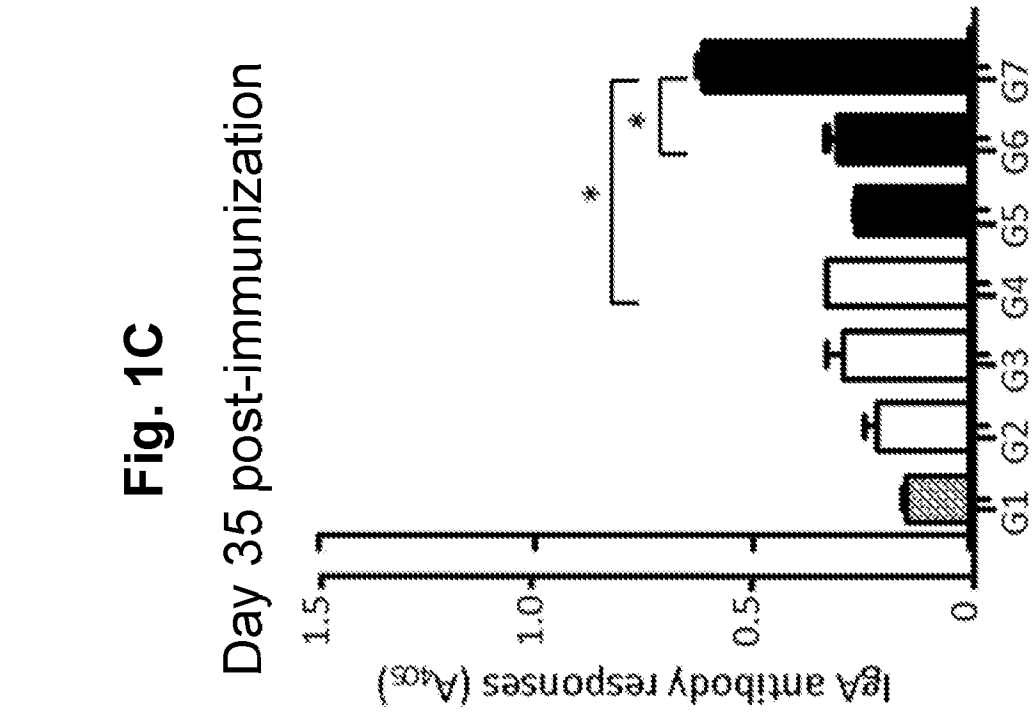

Day 35 post-immunization
IgA antibody responses (A_{405})
1.5
1.0
0.5
0
G1
G2
G3
G4
G5
G6
G7
*
*

Fig. 1D

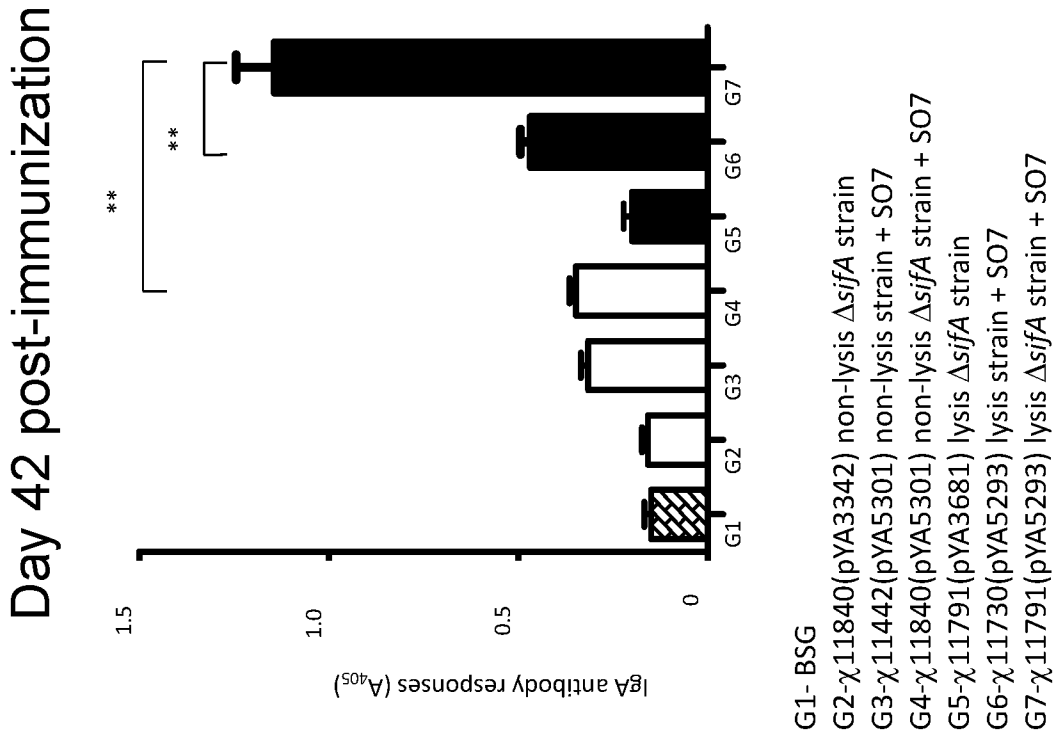
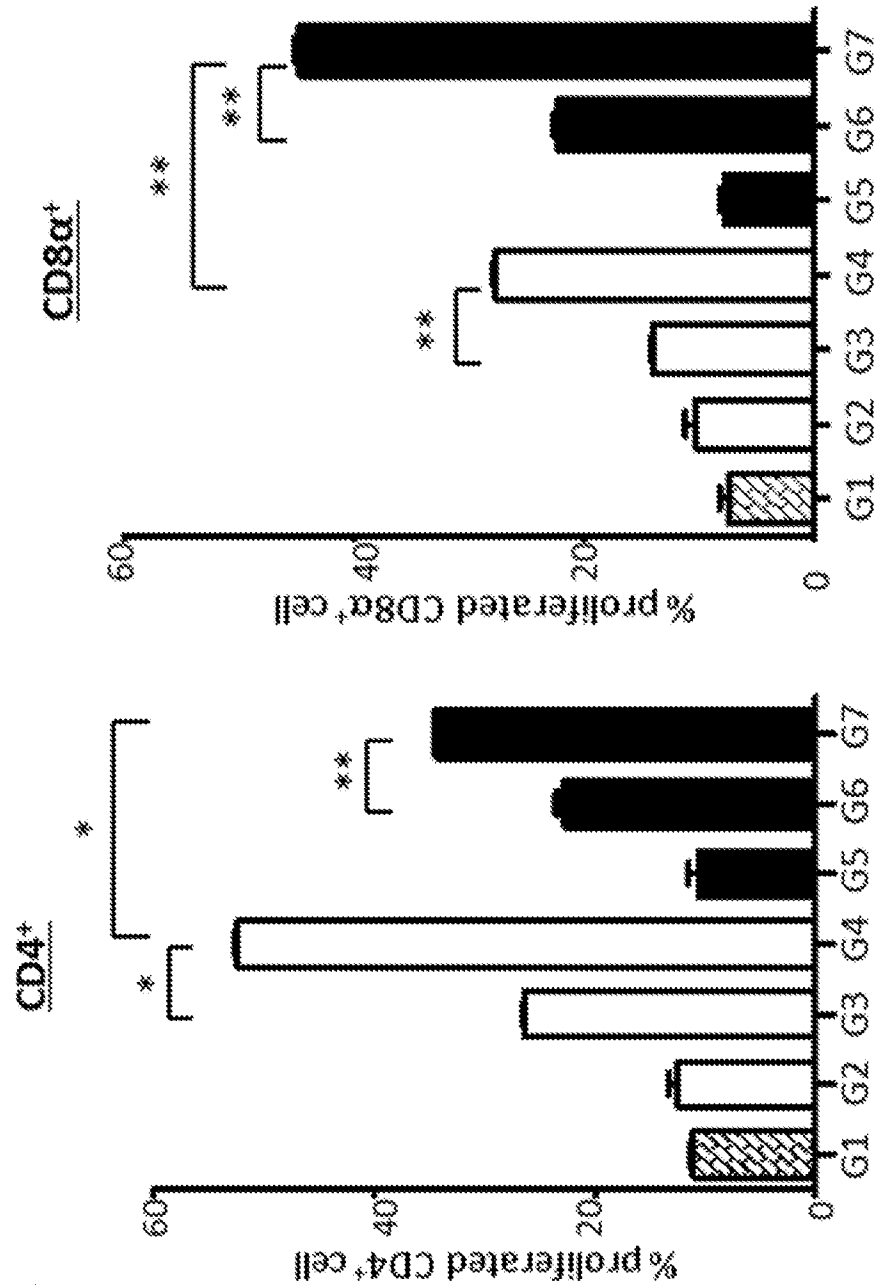
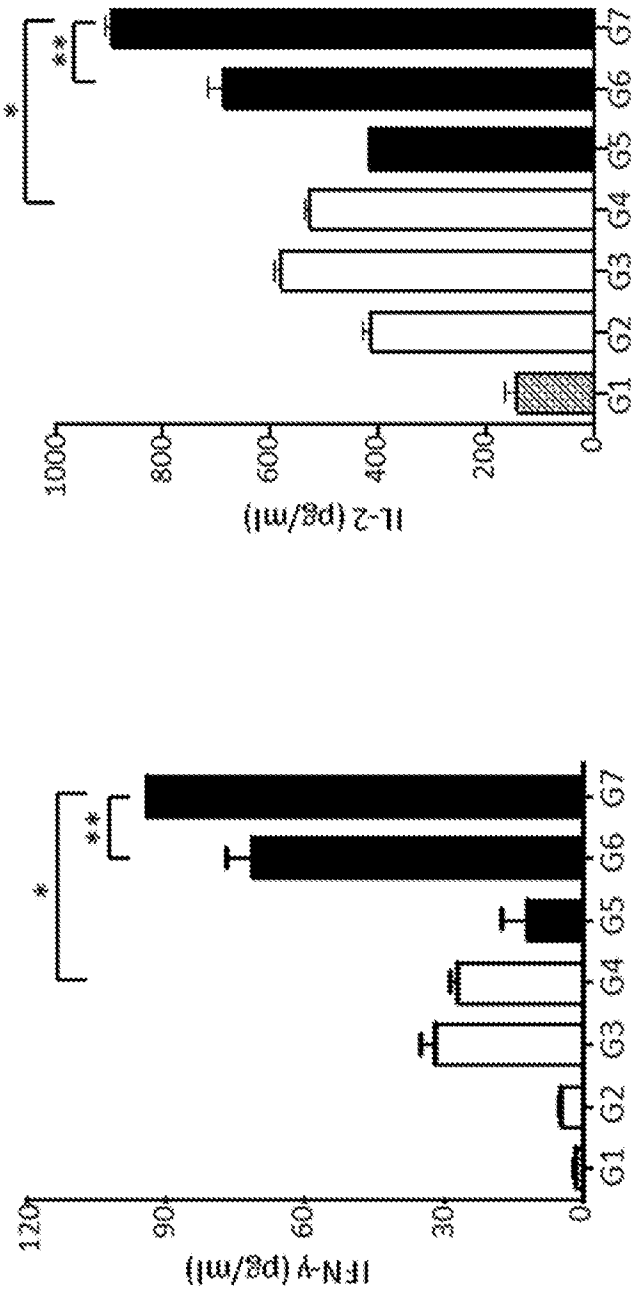

Day 42 post-immunization
IgA antibody responses (A_{405})
1.5
1.0
0.5
0
G1
G2
G3
G4
G5
G6
G7
**
**
G1- BSG
G2-χ11840(pYA3342) non-lysis ΔsifA strain
G3-χ11442(pYA5301) non-lysis strain + SO7
G4-χ11840(pYA5301) non-lysis ΔsifA strain + SO7
G5-χ11791(pYA3681) lysis ΔsifA strain
G6-χ11730(pYA5293) lysis strain + SO7
G7-χ11791(pYA5293) lysis ΔsifA strain + SO7

Fig. 2A



G1- BSG
G2-χ11840(pYA3342) non-lysis Δ*sifA* strain
G3-χ11442(pYA5301) non-lysis strain + SO7
G4-χ11840(pYA5301) non-lysis Δ*sifA* strain + SO7
G5-χ11791(pYA3681) lysis Δ*sifA* strain
G6-χ11730(pYA5293) lysis strain + SO7
G7-χ11791(pYA5293)) lysis Δ*sifA* strain + SO7

Fig. 2B



G1- BSG
G2-χ11840(pYA3342) non-lysis Δ*sifA* strain
G3-χ11442(pYA5301) non-lysis strain + SO7
G4-χ11840(pYA5301) non-lysis Δ*sifA* strain + SO7
G5-χ11791(pYA3681) lysis Δ*sifA* strain
G6-χ11730(pYA5293) lysis strain + SO7
G7-χ11791(pYA5293) lysis Δ*sifA* strain + SO7

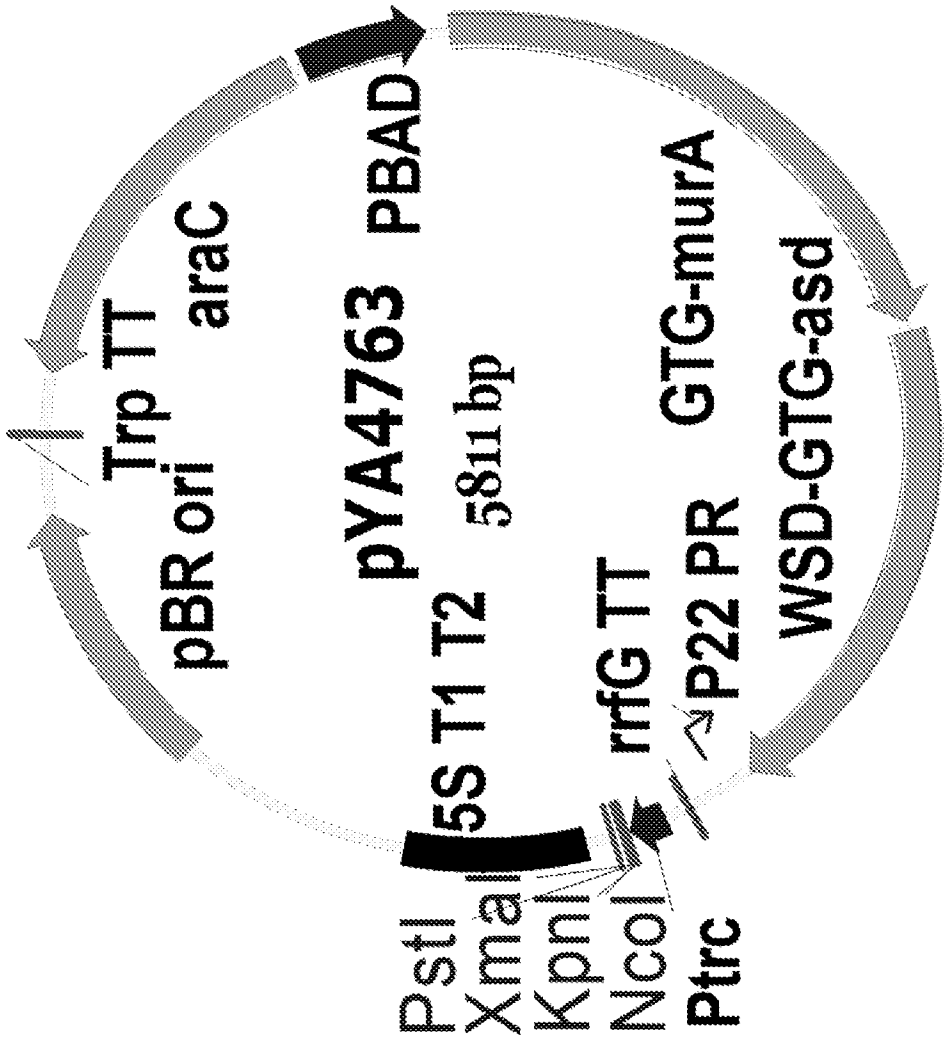

pYA4763
5811 bp
PBAD
araC
Trp TT
pBR ori
5S T1 T2
rrfG TT
P22 PR
GTG-murA
WSD-GTG-asd
PstI
XmaI
KpnI
NcoI
Ptrc

Fig. 3A

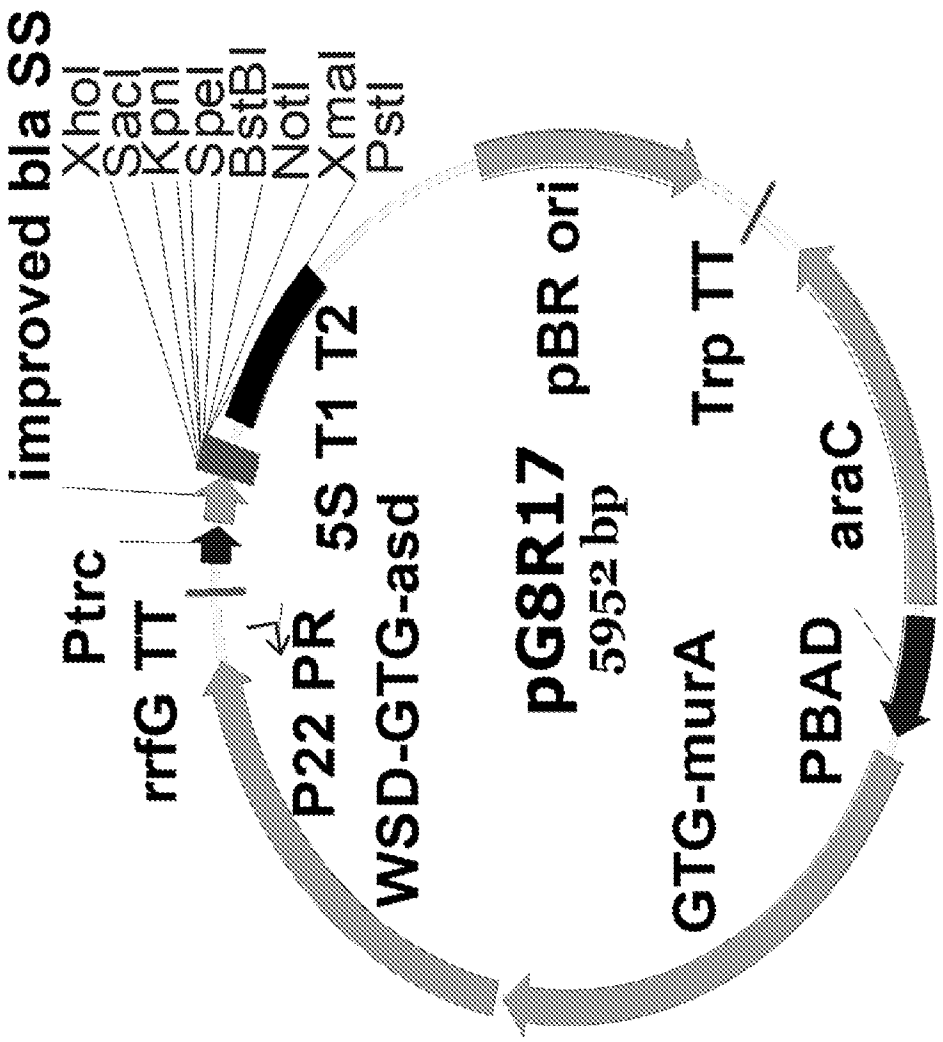

improved bla SS
XhoI
SacI
KpnI
SpeI
BstBI
NotI
XmaI
PstI
Ptrc
rrfG TT
P22 PR
5S T1 T2
WSD-GTG-asd
pG8R17
5952 bp
pBR ori
Trp TT
GTG-murA
PBAD
araC

Fig. 3B

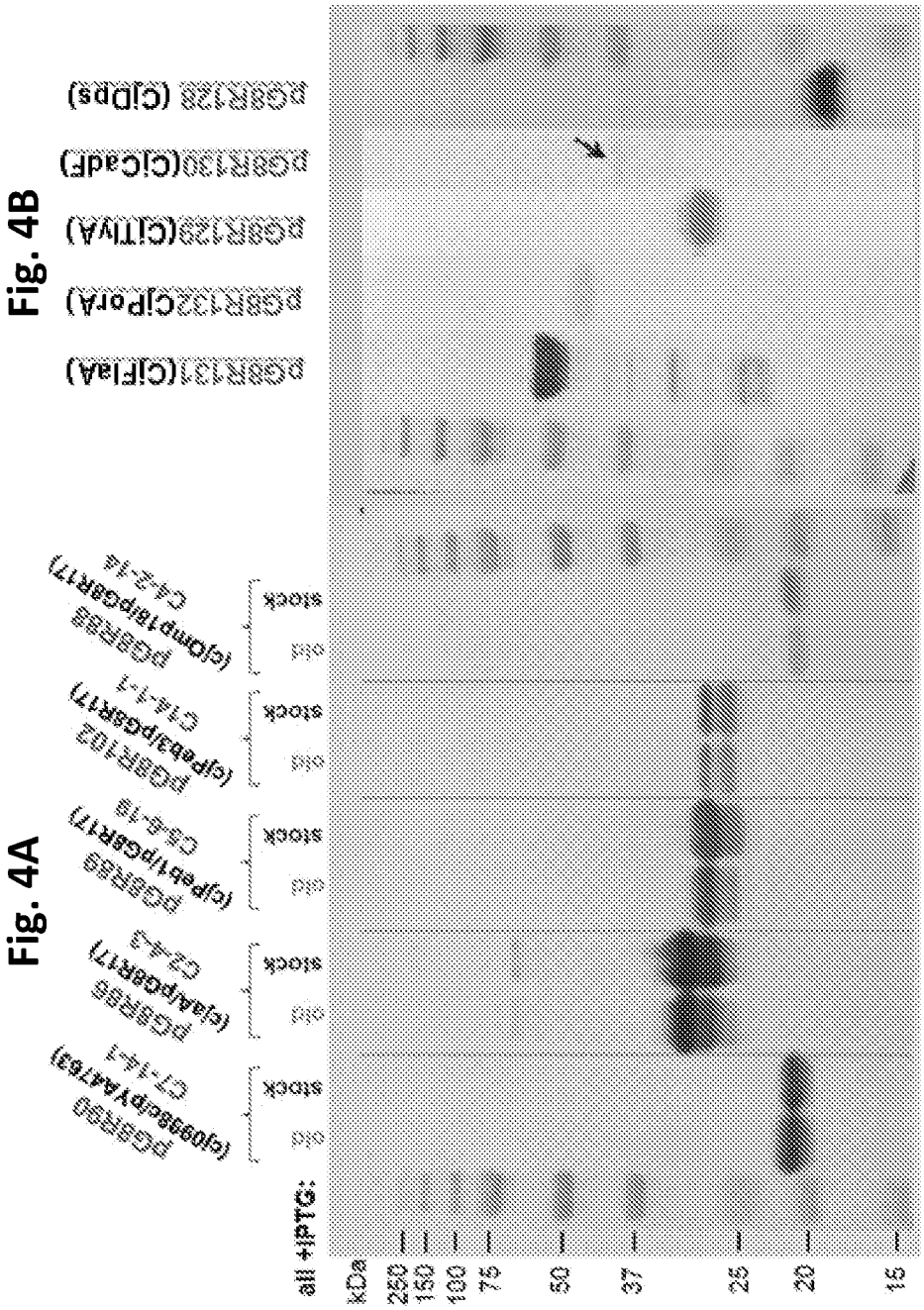
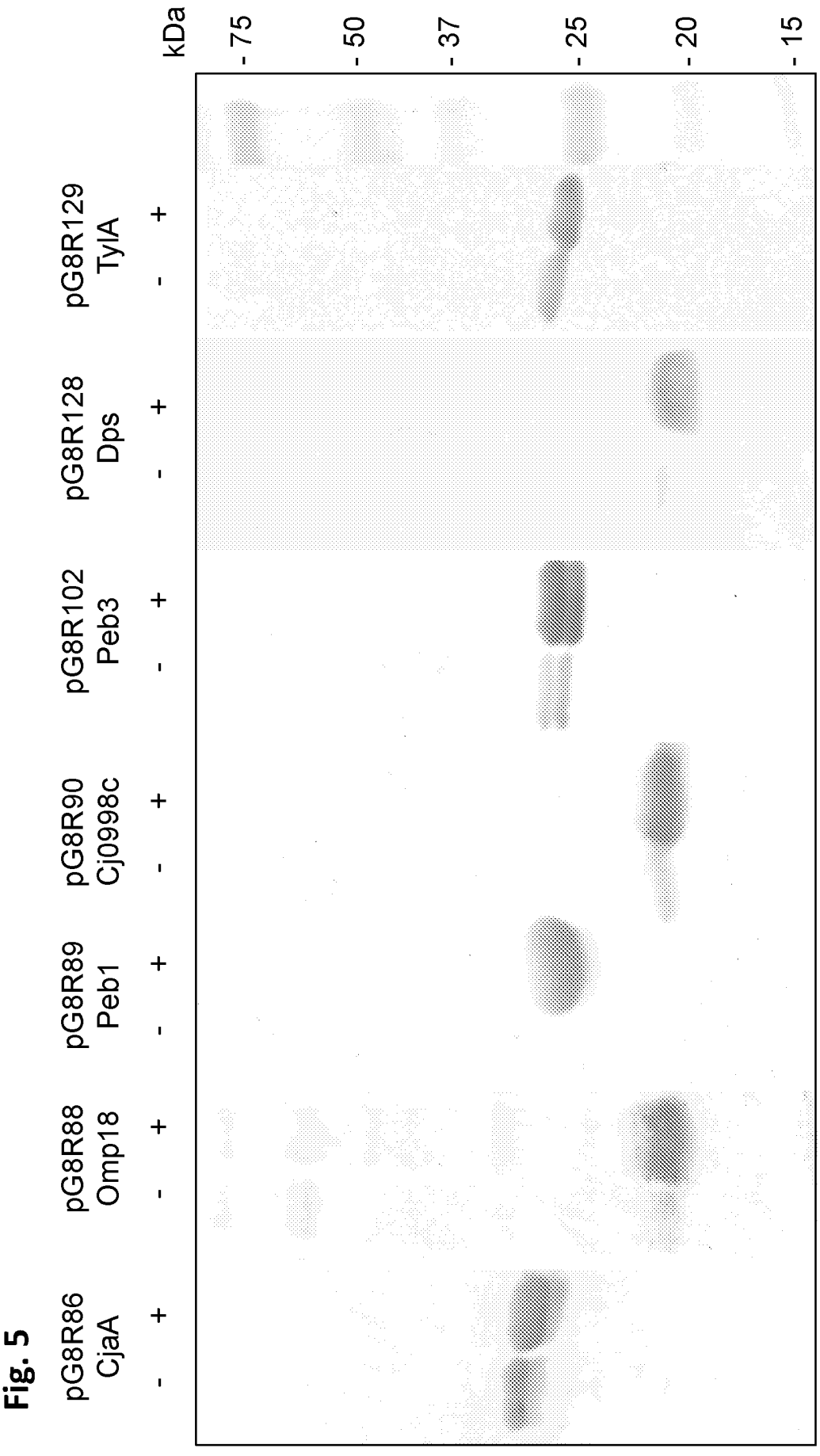

Fig. 4A
Fig. 4B
all +IPTG:
old
stock
kDa
250
150
100
75
50
37
25
20
15
pCSRR5
(clO9Sacl-pVA763)
C7-14-1
pCSRR5
(clJaA/pCSR17)
C2-4-3
pCSRR5
(clPeb1/pCSR17)
C3-6-19
pCSRR102
(clPeb3/pCSR17)
C14-1-1
pCSRR5
(clOmp18/pCSR17)
C4-2-14
pCSR131(ClFlaA)
pCSR132ClPorA)
pCSR129(ClTlvA)
pCSR130(ClcadF)
pCSR128 (ClDps)

Fig. 5


pG8R86 CjaA
pG8R88 Omp18
pG8R89 Peb1
pG8R90 Cj0998c
pG8R102 Peb3
pG8R128 Dps
pG8R129 TylA
kDa
- 75
- 50
- 37
- 25
- 20
- 15

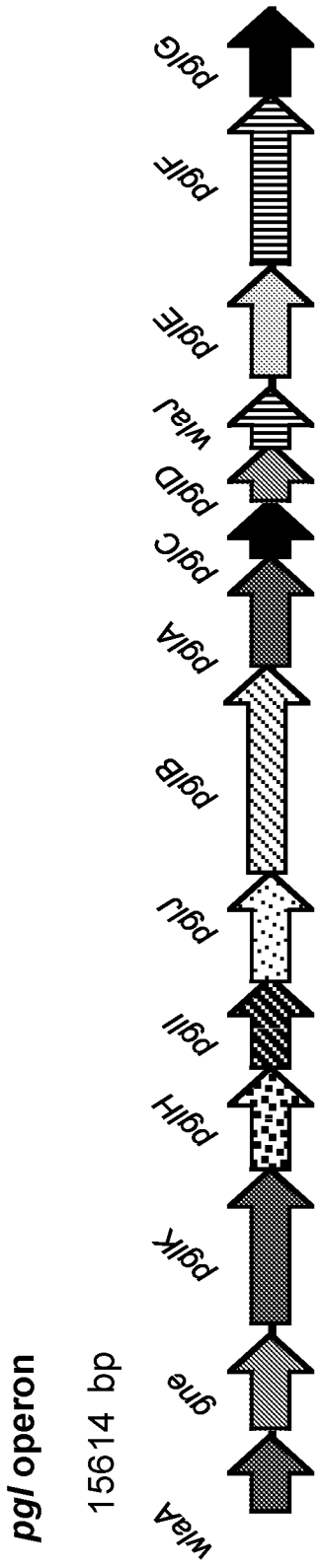

pgl operon
15614 bp
wlaA
gne
pglK
pglH
pglI
pglJ
pglB
pglA
pglC
pglD
wlaJ
pglE
pglF
pglG

Fig. 6

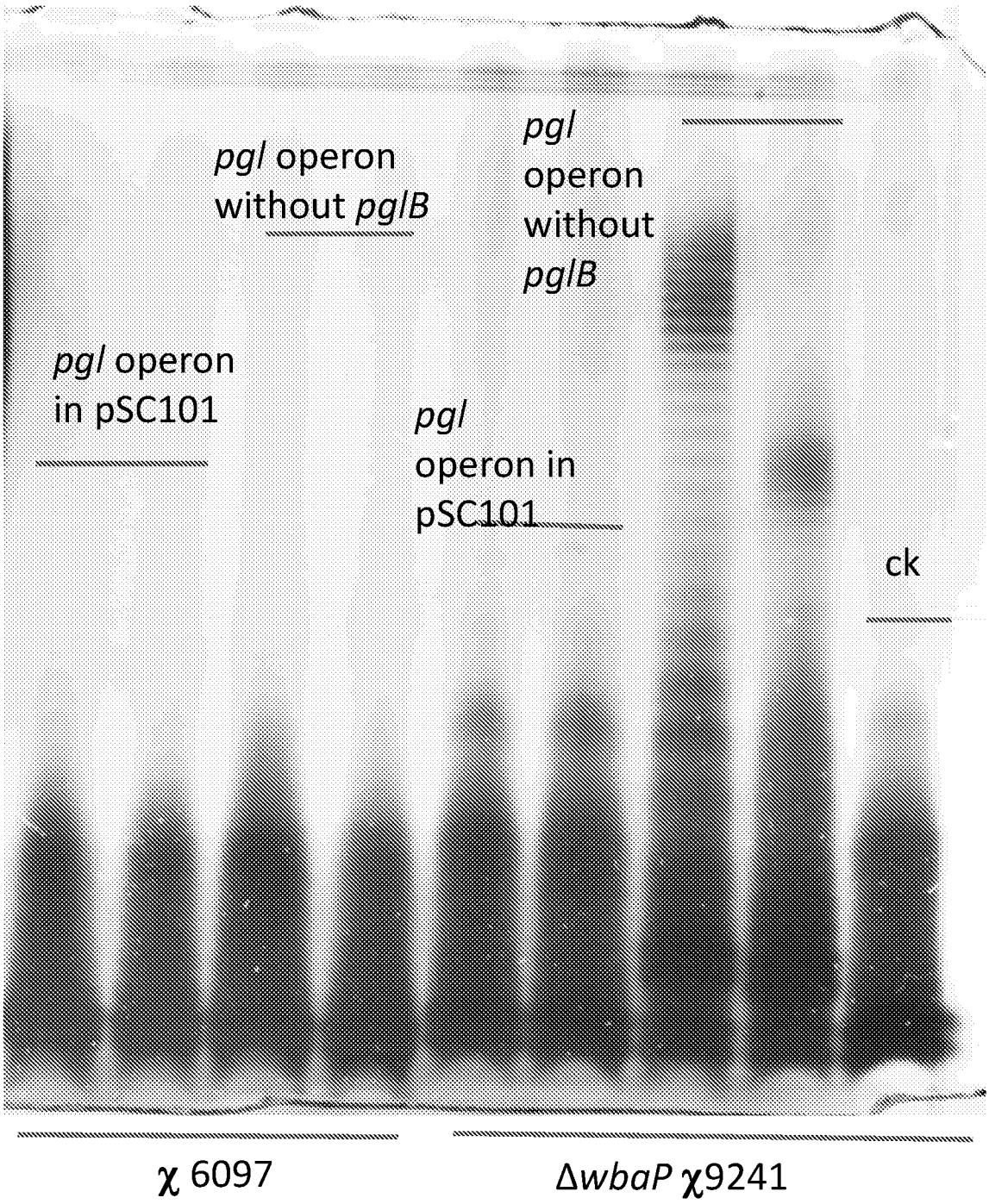
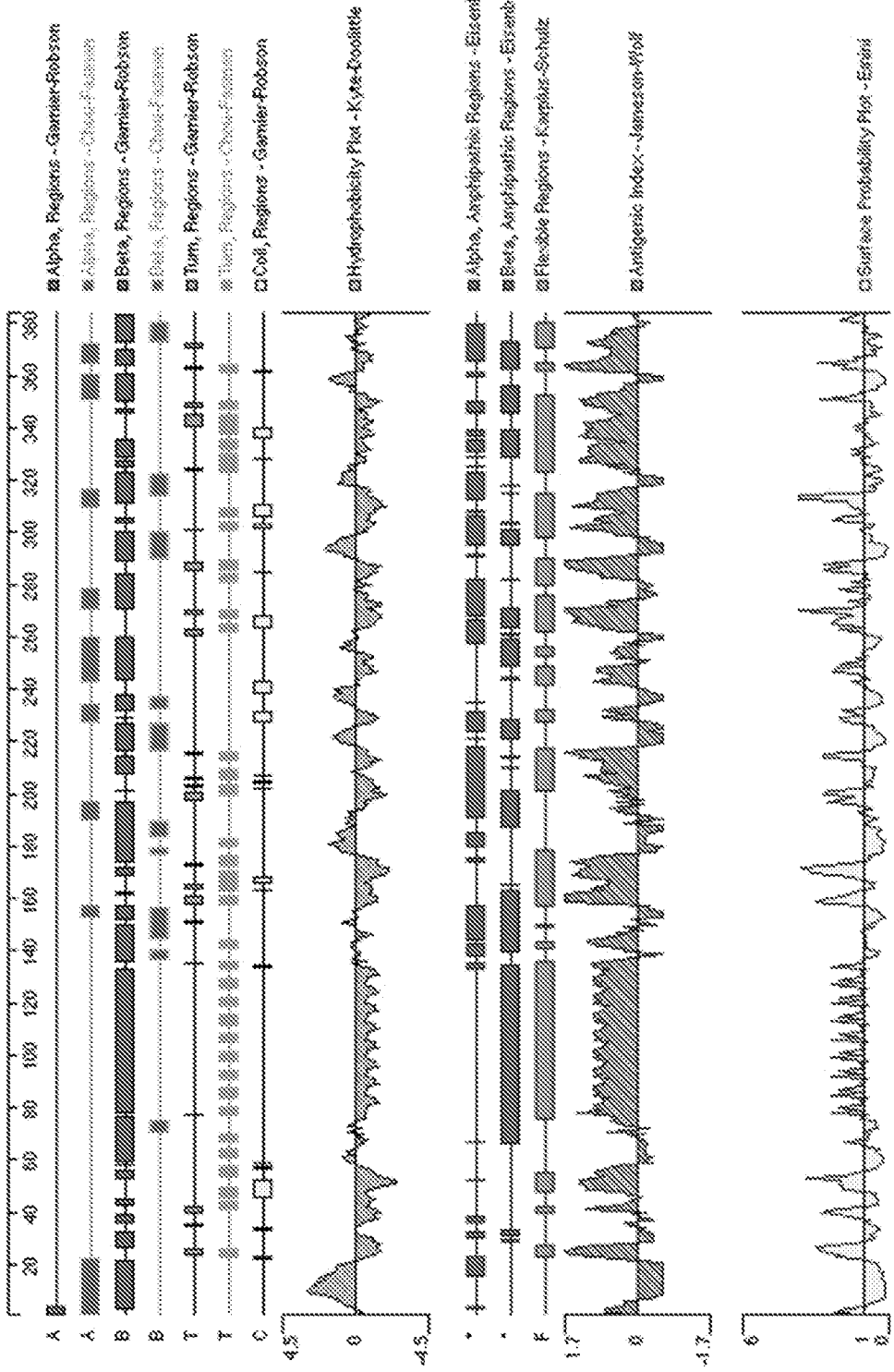

pgl operon without pglB
pgl operon without pglB
pgl operon in pSC101
pgl operon in pSC101
ck
χ 6097
ΔwbaP χ9241

Fig. 7

Fig. 8A: OmpA Δaa70-107 + Cj1443c (aa21-86) insertion
2 beta barrels (3 and 4) of OmpA deleted



Predicted Structural Class of the Whole Protein: Alpha
Deléage & Roux Modification of Nishikawa & Ooi 1987

Analysis	Whole Protein
Molecular Weight	41323.30 m.w.
Length	382
1 microgram =	24.199 pMoles
Molar Extinction coefficient	38640±5%
1 A(280) =	1.07 mg/ml
Isoelectric Point	5.45
Charge at pH 7	-5.31

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	100	31.66	26.18
Acidic (DE)	44	12.63	11.52
Basic (KR)	38	12.53	9.95
Polar (NCQSTY)	113	30.88	29.58
Hydrophobic (AILFWV)	128	32.32	33.51
A Ala	32	5.50	8.38
C Cys	2	0.50	0.52
D Asp	33	9.19	8.64
E Glu	11	3.44	2.88
F Phe	9	3.21	2.36
G Gly	31	4.28	8.12
H His	5	1.66	1.31
I Ile	26	7.12	6.81
K Lys	27	8.37	7.07
L Leu	30	8.22	7.85
M Met	3	0.95	0.79
N Asn	34	9.39	8.90
P Pro	20	4.70	5.24
Q Gln	17	5.27	4.45
R Arg	11	4.16	2.88
S Ser	18	3.79	4.71
T Thr	31	7.58	8.12
V Val	27	6.48	7.07
W Trp	4	1.80	1.05
Y Tyr	11	4.34	2.88
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	1	0.00	0.26

Fig. 8B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/015438

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/015438

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-10, 13-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/015438

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/02; C07K 14/205; C12N 15/74 (2018.01)
CPC - A61K 2039/106; A61K 2039/522; A61K 2039/523; C07K 14/205 (2018.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/200.1; 424/234.1; 530/395 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0199467 A1 (CURTISS, III et al) 14 July 2016 (14.07.2016) entire document	1-6, 11, 12
Y	US 2011/0033501 A1 (CURTISS, III et al) 10 February 2011 (10.02.2011) entire document	1, 2, 6
Y	US 2016/0326563 A1 (ETH ZÜRICH) 10 November 2016 (10.11.2016) entire document	3-6, 11, 12
Y	US 2013/0266604 A1 (SZYMANSKI et al) 10 October 2013 (10.10.2013) entire document	12
A	US 2014/0273163 A1 (GLYCOBIA, INC.) 18 September 2014 (18.09.2014) entire document	1-6, 11, 12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2018	08 MAY 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774