Functional Characterization of Dehydratase/Aminotransferase Pairs from Helicobacter and Campylobacter

**ENZYMES DISTINGUISHING THE PSEUDAMINIC ACID AND BACILLOSAMINE BIOSYNTHETIC PATHWAYS**

Received for publication, October 11, 2005, and in revised form, November 10, 2005
Published, JBC Papers in Press, November 11, 2005, DOI 10.1074/jbc.M511021200

Ian C. Schoenhofen, David J. McNally, Evgeny Vinogradov, Dennis Whitfield, N. Martin Young, Scott Dick, Warren W. Wakarchuk, Jean-Robert Brisson, and Susan M. Logan

From the Institute for Biological Sciences, National Research Council, Ottawa, Ontario K1A OR6, Canada

*Helicobacter pylori* and *Campylobacter jejuni* have been shown to modify their flagellins with pseudaminic acid (Pse), via O-linkage, while *C. jejuni* also possesses a general protein glycosylation pathway (Pgl) responsible for the N-linked modification of at least 30 proteins with a heptasaccharide containing 2,4-diactetamido-2,4,6-trideoxy-α-D-glucopyranose, a derivative of bacillosamine. To further define the Pse and bacillosamine biosynthetic pathways, we have undertaken functional characterization of UDP-α-D-GlcNAc modifying dehydratase/aminotransferase pairs, in particular the *H. pylori* and *C. jejuni* flagellar pairs HP0840/HP0366 and Cj1293/Cj1294, as well as the *C. jejuni* Pgl pair Cj1120c/Cj1121c using His_{het}-tagged purified derivatives. The metabolites produced by these enzymes were identified using NMR spectroscopy at 500 and/or 600 MHz with a cryogenically cooled probe for optimal sensitivity. The enzymes were identified using NMR spectroscopy at 500 and/or 600 MHz with a cryogenically cooled probe for optimal sensitivity. The metabolites of Cj1293 (PseB) and HP0840 (FlaA1) were found to be labile and could only be characterized by NMR analysis directly in aqueous reaction buffer. The Cj1293 and HP0840 enzymes exhibited C6 dehydratase activity as well as a newly identified C5 epimerase activity that resulted in the production of both UDP-2-acetamido-2,6-dideoxy-β-D-arabino-4-hexulose and UDP-2-acetamido-2,6-dideoxy-α-D-xylulose. In contrast, the Pgl dehydratase Cj1120c (PgL) was found to possess only C6 dehydratase activity generating UDP-2-acetamido-2,6-dideoxy-α-D-xylulose. Substrate-specificity studies demonstrated that the flagellar aminotransferases HP0366 and Cj1294 utilize only UDP-2-acetamido-2,6-dideoxy-β-D-arabino-4-hexulose as substrate producing UDP-4-amino-4,6-dideoxy-β-D-altrose, a precursor in the Pse biosynthetic pathway. In contrast, the Pgl aminotransferase Cj1121c (PglE) utilizes only UDP-2-acetamido-2,6-dideoxy-α-D-xylulose producing UDP-4-amino-4,6-dideoxy-α-D-GlcNAc (UDP-2-acetamido-4-amino-2,4,6-trideoxy-α-D-glucopyranosamine), a precursor used in the production of the Pgl glycan component 2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranosamine.

*Campylobacter jejuni*, a principle cause of acute gastroenteritis, and *Helicobacter pylori*, a major etiological agent of gastroduodenal disease, are microaerophilic Gram-negative bacteria (1, 2). As these organisms have significant medical and public health importance, recent genomic efforts have resulted in the complete sequencing of at least two genomes for each organism with others in progress (3–6). The sequencing of *C. jejuni* genomes revealed an unusual plethora (>8%) of glycan biosynthetic genes dedicated to surface carbohydrate biosynthesis, as well as significant diversity of glycan biosynthetic gene content among individual strains (7). These gene products appear to be involved in the biosynthesis of the glycoconjugate structures lipooligosaccharide and capsular polysaccharide, as well as in the biosynthesis of the novel glycans utilized by the N- and O-linked protein glycosylation systems. Each respective glycoconjugate structure is represented by a distinct and dedicated genetic locus (lipooligosaccharide Cj1131-Cj1152, capsule Cj1448c-Cj1413c, N-linked glycan Cj1119-Cj1130, O-linked glycan Cj1293-Cj1342). Although much progress has been made toward biosynthetic and structural characterization of lipooligosaccharide, capsular polysaccharide, and N-linked systems, the biochemical characterization of the O-linked system is currently less well defined (7–9).

*C. jejuni* has been shown to modify its flagellin with the novel nine-carbon sialic acid-like sugar 5,7-diactetamido-3,5,7,9-tetra(deoxy)-L-glycerol-1-manno-nonulosonic acid or pseudaminic acid (Pse), as well as related derivatives, via O-linkage at up to 19 sites/monomer (10, 11). The modification of flagellin appears to be important for flagellar assembly, as mutations in putative O-linked glycosylation genes result in non-motile cells lacking flagella (11). *C. jejuni* also possesses a general protein glycosylation pathway (Pgl) that is responsible for the N-linked addition of a heptasaccharide containing N-acetylgalactosamine, glucose, and 2,4-diactetamido-2,4,6-trideoxy-α-D-glucopyranosamine (2,4-diactetamido-Bac) to at least 30 different proteins (12, 13). Interruption of either the flagellin glycosylation or Pgl pathway results in loss of colonization and, hence, virulence (14, 15); as such, the Pse and Bac biosynthetic pathways offer potential as novel therapeutic targets. Although biosynthesis of both the complex carbohydrates Bac and Pse from the initial N-acetyl-hexosamine building block, UDP-α-D-GlcNAc, would involve the actions of a sugar-nucleotide dehydratase and its corresponding aminotransferase, the precise biosynthetic steps that distinguish these pathways are currently unknown.

8 This study was supported by the National Research Council Genomics and Health Initiative. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 The abbreviations used are: Pse, 5,7-diactetamido-3,5,7,9-tetra(deoxy)-L-glycerol-1-manno-nonulosonic acid (also known as pseudaminic acid); AltnAc, N-acetyl-β-D-altroside; Bac, 2,4-diactetamido-2,4,6-trideoxy-α-D-glucopyranosamine (also known as bacillosamine); CE, capillary electrophoresis; δ, chemical shift; HMBC, heteronuclear multiple bond coherence; HSQC, heteronuclear single quantum coherence; j, proton-coupling constant; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; Pgl, general protein glycosylation pathway; PLP, pyridoxal phosphate; TOCSY, total correlation spectroscopy.
The flagellins of *H. pylori* have also been shown to be modified with Pse, where glycosylation again appears to be required for assembly of a functional filament (16). Homologs of *Campylobacter* flagellar glycosylation components are present in both the *H. pylori* 26695 and the J99 genomes (5, 6). Metabolic analysis of various O-linked glycan biosynthetic mutants revealed an accumulation of biosynthetic intermediates and loss of CMP-Pse, thus confirming a role for these gene products in the Pse biosynthetic pathway, although the precise enzymatic steps of this pathway remain ill defined (16). To unequivocally define the Bac and Pse biosynthetic pathways, we have undertaken functional characterization of the *C. jejuni* and *H. pylori* flagellar pairs Cj1293/Cj1294 and HP0840/HP0366, as well as the *C. jejuni* Pgl pair Cj1120C/Cj1121C, using His$_6$-tagged recombinant proteins. These results convincingly demonstrate that the Pse and Bac biosynthetic pathways are discrete, incorporating unique and distinct dehydratase/aminotransferase pairs, and explain the clear phenotypic differences observed between isogenic mutants from the two pathways.

**EXPERIMENTAL PROCEDURES**

**DNA Techniques and Plasmid Construction**—Plasmid DNA mini-preparations and agarose gel purification of DNA fragments were performed using Qiagen’s QIAprep spin kit and QIAquick gel extraction kit, respectively. All other recombinant DNA methods and analyses were performed as described by Sambrook et al. (17). Vector or recombinant plasmids were transformed by electroporation into electrocompetent Top10F$^+$ or DH10B (Invitrogen) *E. coli* cells for cloning purposes or BL21[DE3] (Novagen, Madison, WI) *E. coli* cells for protein production. The polymerase chain reaction (PCR) was used to amplify *H. pylori* 26695 DNA or *C. jejuni* 11168 DNA for subsequent cloning. A list of cloning vectors and recombinant plasmids is provided in Table 1, and pertinent oligos are provided in Table 2. PCR was performed in a 50-µl reaction containing 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl$_2$, dNTPs at a final concentration of 200 µM, 0.2 µM of each primer, and 2.5 units of Taq DNA polymerase (Roche Applied Science). Amplicons were ligated with pCR2.1 and the agarose-purified restriction fragment was cloned into the pCR2.1 and pCR2.1 Ap r,K nr, oriColE1, T7 promoter, used for N-terminal His$_6$-tagged protein expression. pCR2.1, pCR2.1 Ap r,K nr, oriColE1, T7 promoter, used for C-terminal His$_6$-tagged protein expression. pET30a, pET15b derivative; Ap r, oriColE1, T7 promoter, used for N-terminal His$_6$-tagged protein expression. This study

**Biosynthetic Enzymes of the Pse and Bac Pathways**

| Plasmid     | Description                                                                 | Source/Reference |
|-------------|-----------------------------------------------------------------------------|------------------|
| pCR2.1      | Ap$^+$, Kan$^+$, oriColE1, lac promoter, used for TA cloning                  | Invitrogen       |
| pET30a      | pET15b derivative; Ap$^+$, oriColE1, T7 promoter, used for C-terminal His$_6$-tagged protein expression | This study       |
| pFO4        | pET15b derivative; Ap$^+$, oriColE1, T7 promoter, used for N-terminal His$_6$-tagged protein expression | Novagen          |
| pNRC8.1     | HP0840 NdeI-Xhol in pET30a, encodes for HP0840His$_{pseC}$                   | This study       |
| pNRC37.1    | HP0366 BamHI-EcoRI in pFO4, encodes for His$_6$HP0366                       | This study       |
| pNRC20.3    | Cj1293 NdeI-Xhol in pET30a, encodes for Cj1293His$_{pseC}$                   | This study       |
| pNRC28.1    | Cj1294 BamHI-EcoRI in pFO4, encodes for His$_6$Cj1294                       | This study       |
| pNRC40.1    | Truncated Cj120C BamHI-EcoRI in pFO4, encodes for His$_6$Cj120C              | This study       |
| pNRC41.3    | Cj1121C BamHI-EcoRI in pFO4, encodes for His$_6$Cj1121C                     | This study       |

**TABLE 1**

Plasmids used in this study

| Plasmid     | Description                                                                 | Source/Reference |
|-------------|-----------------------------------------------------------------------------|------------------|
| pCR2.1      | Ap$^+$, Kan$^+$, oriColE1, lac promoter, used for TA cloning                  | Invitrogen       |
| pET30a      | pET15b derivative; Ap$^+$, oriColE1, T7 promoter, used for C-terminal His$_6$-tagged protein expression | This study       |
| pFO4        | pET15b derivative; Ap$^+$, oriColE1, T7 promoter, used for N-terminal His$_6$-tagged protein expression | Novagen          |
| pNRC8.1     | HP0840 NdeI-Xhol in pET30a, encodes for HP0840His$_{pseC}$                   | This study       |
| pNRC37.1    | HP0366 BamHI-EcoRI in pFO4, encodes for His$_6$HP0366                       | This study       |
| pNRC20.3    | Cj1293 NdeI-Xhol in pET30a, encodes for Cj1293His$_{pseC}$                   | This study       |
| pNRC28.1    | Cj1294 BamHI-EcoRI in pFO4, encodes for His$_6$Cj1294                       | This study       |
| pNRC40.1    | Truncated Cj120C BamHI-EcoRI in pFO4, encodes for His$_6$Cj120C              | This study       |
| pNRC41.3    | Cj1121C BamHI-EcoRI in pFO4, encodes for His$_6$Cj1121C                     | This study       |

Grown in 500 ml of 2x yeast tryptone (17) with either kanamycin (50 µg ml$^{-1}$) or ampicillin (50 µg ml$^{-1}$) for selection. The cultures were grown at 30 °C, induced at an OD$_{600}$ of 0.6 with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside, and harvested 2.75 h later. Cell pellets were resuspended in lysis buffer (50 mM sodium phosphate, pH 7.7, 400 mM NaCl, 10 mM β-mercaptoethanol) containing 10 mM imidazole and complete protease inhibitor mixture, EDTA-free (Roche Applied Science). After addition of 10 µg ml$^{-1}$ of RNaseA and DNaseI (Roche Applied Science), the cells were disrupted by two passes through an emulsiflex C5 (20,000 psi). Lysates were centrifuged at 100,000 × $g$ for 1 h at 4 °C, and the supernatant fraction was applied to a 1-ml nickel-nitrilotriacetic acid (Qiagen) column equilibrated in 10 mM imidazole lysis buffer, using a flow rate of 0.5 ml min$^{-1}$. After sample application, the column was washed with 20 column volumes of 10 mM imidazole Lysis buffer. To elute the protein of interest, a linear gradient from 10 to 100 mM imidazole, in lysis buffer, over 60 column volumes was applied to the column prior to a final pulse of 40 column volumes of 200 mM imidazole lysis buffer. Fractions containing the purified protein of interest, as determined by SDS-PAGE (12.5%) and Coomassie staining, were pooled and dialyzed against dialysis buffer (25 mM sodium phosphate, pH 7.7, 50 mM NaCl) overnight at 4 °C. Protein concentration was measured spectrophotometrically using $A_{280}$ 0.1% values (HP0840His$_{pseC}$ 0.536; His$_6$HP0366, 0.386; Cj1293His$_{pseC}$ 0.669; His$_6$Cj1294, 0.635; His$_6$SFCj1120C, 0.410; His$_6$Cj1121C, 0.902).

**Enzymatic Reactions**—Purification and dialysis/assay buffers for flagellar glycosylation enzymes were pH 7.2, whereas those for Pgl enzymes were pH 7.7. HP0840His$_{pseC}$, Cj1293His$_{pseC}$, and His$_6$SFCj1120C reactions were scaled up to a total reaction volume of 20 ml containing 6 mg of enzyme in the presence of 1 mM UDP-α-D-GlcNAc. Time course samples were also taken for these dehydratase reactions at the times indicated; the samples were then boiled for 3 min, centrifuged for 3 min, and diluted 1:4 in H$_2$O prior to capillary electrophoresis (CE) analysis. For the HP0840His$_{pseC}$/HP0366 and the Cj1293His$_{pseC}$/His$_6$Cj1294 coupled assays, the reactions were scaled up to a total volume of 30 ml containing 9 mg of each enzyme in the presence of 1 mM UDP-α-D-GlcNAc, 10 mM L-Glu, and 1 mM pyridoxal phosphate (PLP) (the latter two being cofactors necessary for the aminotransfer reaction). To prepare the His$_6$Cj1121C product, 25 ml of a His$_6$SFCj1120C reaction containing 7.5 mg of enzyme in the presence of 1 mM UDP-α-D-GlcNAc was allowed to proceed for 210 min. After passage through an Amicon Ultra-15 (10,000 molecular weight cut-off) filter membrane, 6.3 mg of His$_6$Cj1121C was added to the filtrate, along with PLP and L-Glu, to a final concentration of 1 and 10 mM, respectively. After incubation at 37 °C for 210 min, all reaction mixtures were passed through filter membranes as described above. The filtrates were then either 1) lyophilized and desalted using a Bio-Gel P-2 (Bio-Rad) column in 50 mM ammonium bicarbonate, pH 7.8, as well as a Sephadex G-15 column in pyridine/acetic acid:H$_2$O (1:2.5:250) prior to NMR, or 2) analyzed directly by NMR in aqueous reaction buffer. Sample composition (i.e.
distribution of products II-V, see Fig. 4) was analyzed by CE prior to NMR analysis.

Analysis of Enzymatic Reaction Products by NMR Spectroscopy—Purified reaction products were suspended in 200 μl of 99% deuterated water (Cambridge Isotopes Laboratories Inc.). For examination of metabolites immediately after an enzymatic reaction was completed, the sample (90% H₂O/10% deuterated water) was prepared by adding 20 μl of 99% deuterated water to 180 μl of reaction mixture. All samples were placed into 3-mm NMR tubes. Standard homo- and heteronuclear-related two-dimensional 1H NMR, 13C NMR, 15N HSQC, 31P HSQC, HMBC, COSY, TOCSY, NOESY pulse sequences from Varian (Varian, Palo Alto, CA) were used for general assignments. Selective one-dimensional TOCSY, with a Z-filter, and NOESY experiments were used for complete residue assignment and measurement of proton-coupling constants, 1H, 13C, 15N,H,H, and NOEs (18, 19). The analysis of 1H, 13C, and 15N values for various pyranose chair conformations was based on the coordinates for ideal pyranose chair conformations (20). NMR experiments were performed with a Varian 600 MHz (1H) spectrometer equipped with a Varian 5-mm Z-gradient triple resonance (1H, 13C, 15N) cryogenically cooled probe (cold probe) and with a Varian Inova 500 MHz (1H) spectrometer with a Varian Z-gradient 3-mm triple resonance (1H, 13C, 31P) probe. Direct detected 31P NMR spectra were acquired using a Varian Mercury 200 MHz (1H) spectrometer with a Nalorac 5-mm four nuclei probe. NMR experiments were typically performed at 25 °C with suppression of the H₂O or deuterated HOD resonance at 4.78 ppm. For proton and carbon experiments, the methyl resonance of acetone was used as an internal reference (δC 31.07 ppm), and an external 85% phosphoric acid standard (δP 0 ppm) was used to reference 31P spectra.

Analysis of Reaction Products by CE—CE analysis was performed using a P/ACE 5510 system (Beckman Instruments) with diode array detection. The running buffer was 25 mM sodium tetraborate, pH 9.4. The capillary was either bare silica 50 μm × 50 cm or 75 μm × 50 cm, with a detector at 50 cm. The capillary was conditioned before each run by washing with 0.2 M NaOH for 2 min, water for 2 min, and running buffer for 2 min. Samples were introduced by pressure injection for 6–10 s, and the separation was performed at 18 kV for 20 min. Peak integration was done using the Beckman P/ACE station software.

Kinetic Measurements of His6Cj1294 and His6Cj1121c—The substrates for the aminotransferases, UDP-2-acetamido-2,6-dideoxy-β-L-arabinono-4-hexulose and UDP-2-acetamido-2,6-dideoxy-α-D-xylono-4-hexulose, were prepared by incubating UDP-α-D-GlcNAc with Cj1293His6. This substrate preparation was used because of its versatility in studying both Pse and Bac aminotransferases. To determine the preferred substrate for His6Cj1294 and His6Cj1121c, −15 μg of each enzyme was incubated with this Cj1293His6 filtrate, containing 10 mM L-Glu and 1 mM PLP, and subjected to time course analysis (see above). For kinetic analyses, 5 μg of His6Cj1294 in a total volume of 200 μl or 0.15 μg of His6Cj1121c in a total volume of 400 μl was incubated at 37 °C in dialysis buffer, pH 7.2, containing 1 mM PLP, 10 mM L-Glu, and various quantities of UDP-2-acetamido-2,6-dideoxy-β-L-arabinono-4-hexulose and UDP-2-acetamido-2,6-dideoxy-α-D-xylono-4-hexulose. His6Cj1121c assays also contained 40 μg of acetylated bovine serum albumin as carrier protein. Substrate conversion was determined using the molar extinction coefficient of UDP (ε260 = 10,000). Kinetic constants were calculated using Eadie-Hofstee plots with the program GraphPad Prism 3.

RESULTS

Protein Expression and Purification—In this study, C. jejuni protein-coding sequences were cloned from C. jejuni 11168 DNA, and H. pylori sequences were cloned from strain 26695. Sugar nucleotide-modifying dehydratases were sensitive to N-terminal modification, whereas derivatives containing His6 tags located at the C terminus exhibited greater stability and solubility compared with their N-terminal counterparts (data not shown). As such, Cj1293 and its functional homolog HP0840 from H. pylori were both constructed with C-terminal His6 tags. In contrast, the Cj1120c derivative was designed with an N-terminal His6 tag, although, as described below, this was not adjacent to the catalytic domain. This derivative is a soluble truncated version of the native Cj1120c membrane protein, encoding only residues 130–590. From sequence alignment with the short soluble family of dehydratases the Cj1120c polypeptide can be divided into three domains: an N-terminal domain, residues 130–590 (data not shown). The Cj1120c derivative was designed with an N-terminal His6 tag, although, as described below, this was not adjacent to the catalytic domain. This derivative is a soluble truncated version of the native Cj1120c membrane protein, encoding only residues 130–590. From sequence alignment with the short soluble family of dehydratases the Cj1120c polypeptide can be divided into three domains: an N-terminal domain, residues 130–590, and a catalytic domain, residues 129–130 (data not shown). To assess the function of Cj1120c under native state conditions, the linker region was retained, although a construct containing only residues 189–590 exhibited similar activity to that observed with the 130–590 construct (data not shown). In contrast to the dehydratases, the aminotransferases HP0366, Cj1294, and Cj1121c were more resistant to N-terminal modification, but soluble yields were lower compared with the dehydratases (data not shown).

Overall, yields of −20 mg/liter starting culture were obtained for all of the proteins tested, which had near homogeneity purity (Fig. 1). Protein production levels, as well as the correlation between size estimates determined by SDS-PAGE and that predicted from sequence, confirmed the identity of each purified His6-tagged protein.

### TABLE 2

**Oligonucleotides used in this study**

| Oligonucleotide | Sequence (5′ → 3′) | Purpose |
|-----------------|-------------------|--------|
| NRC21           | CATATGCAAAAATCATCAAAAAATGCTAGAC | Cloning of pNRC8.1 |
| NRC22           | CTGAGTAATAATTTTAGCTATGCAGGCTC | Cloning of pNRC37.1 |
| NRC60           | GGATCCGCTAAGAGTGGTTCGTTATAG | Cloning of pNRC20.3 |
| NRC61           | AAAGAATCTTCCTTTTATTTTAAACACTCAAAG | Cloning of pNRC82.1 |
| NRC43           | CATATGTTAATACAAAAAAATATCTTATACGGGG | Cloning of pNRC20.3 |
| NRC94           | CTCGAGAAAAACCTTCTGATGTAGTAGTTGTTTTC | Cloning of pNRC20.3 |
| NRC137          | GGATCCGCTTCTATCTTCTCATCAAAAAATCAC | Cloning of pNRC40.1 |
| NRC138          | GAATTCTATTGAACATTTGATTTTAAACCTAATTC | Cloning of pNRC41.3 |
| NRC81           | GAATCCGATGTTGGATTTTAAACCTTCTC | Sequencing of pET30a constructs |
| NRC74           | GAATCTTTAATAGCCTTTTATTTTAAATTTC | Sequencing of pET30a constructs |
| NRC75           | GAATCCGATGTTATTGATTTTAAACCTTCTC | Sequencing of pET30a constructs |
| NRC76           | GAATCTTTAAGGCTTATTTGCTTATTAGATC | Sequencing of pET30a constructs |
| T7-F            | TAAATACGACTCACTATAGGG | Sequencing of pET30a constructs |
| T7-R            | GCTATTTAGGCTTACCGG | Sequencing of pET30a constructs |
| NRC175          | TTAATACGACTCACTATAGGG | Sequencing of pET30a constructs |
| NRC160          | GGTTATGCTAGTTATGCTACGG | Sequencing of pET30a constructs |

**Biosynthetic Enzymes of the Pse and Bac Pathways**

---

**JANUARY 13, 2006 • VOLUME 281 • NUMBER 2**

**JOURNAL OF BIOLOGICAL CHEMISTRY**

725
NAD(P)ases studied here did not require the addition of exogenous cofactor His6SFCj1120c; were identical to those previously reported for UDP-as the proton-coupling constants determined for product II (Table 3), the enzymes and was recycled throughout catalysis. The slower reaction product formed by both enzymes is the UDP-4-hexulose or UDP-2-acetamido-2,6-dideoxy-4-hexulose sugar (see below) was also obtained within a comparatively short time of Cj1293His6/His6Cj1294 displayed similar CE mobility compared with the HP0840His6/His6HP0366 product (Fig. 4, lane 3). The selective TOCSY for H-6 facilitated the assignment of H-5 (data not shown). Accurate proton-coupling constants were measured from the TOCSY spectra (Table 3). The selective NOESY of IV H-1 revealed NOEs to H-2 and H-5 (Fig. 3c), whereas the selective NOESY of IV H-6 showed NOEs to H-3 and H-5 (Fig. 3d). The proton-coupling constants and NOEs were indicative of an arabino-4-hexulose sugar (see below) (supplemental Table S2). The 13C chemical shifts were obtained from the HSQC spectrum (Fig. 3f). The proton-coupling constants and NOEs indicated the sugar ring was flexible. The 31P HSQC spectrum and assignments for uridine and ribose confirmed that these were UDP sugars. The use of the cold probe facilitated the assignment of carbon chemical shifts for the highly unstable UDP-arabinio-sugar as the 13C HSQC spectrum was obtained in a few hours (Fig. 3f). HMBC spectra (data not shown) were also obtained within a comparatively short time span using the cold probe and were pivotal in assigning the quaternary C-4 carbons for both II and IV.

CE Characterization of HP0366, Cj1294, and Cj1121c Aminotransferase Products—CE was used to classify the reaction products generated by the dehydratases and aminotransferases as belonging to either the Pse or Bac biosynthetic pathways (Fig. 4). Here, it is shown that the Pse dehydratases HP0840 and Cj1293, as well as the Bac dehydratase Cj1120c, produce the UDP-2-acetamido-2,6-dideoxy-α-D-arabino-4-hexulose intermediate after 3.5 h. It should be noted, however, that the initial product of the Pse dehydratases is UDP-2-acetamido-2,6-dideoxy-β-L-arabinio-4-hexulose or UDP-arabinio-sugar (IV) (Fig. 3 and supplemental Fig. S2). Both metabolites could clearly be distinguished in the 1H and 13C HSQC spectrum (Fig. 3, a and f). The selective TOCSY of IV H-1 permitted the assignment of H-2 and H-3 (Fig. 3b), and the selective TOCSY for H-6 facilitated the assignment of H-5 (data not shown). Accurate proton-coupling constants were measured from the TOCSY spectra (Table 3). The selective NOESY of IV H-1 revealed NOEs to H-2 and H-5 (Fig. 3c), whereas the selective NOESY of IV H-6 showed NOEs to H-3 and H-5 (Fig. 3d). The proton-coupling constants and NOEs were indicative of an arabino-4-hexulose sugar (see below) (supplemental Table S2). The 13C chemical shifts were obtained from the HSQC spectrum (Fig. 3f). The proton-coupling constants and NOEs indicated the sugar ring was flexible. The 31P HSQC spectrum and assignments for uridine and ribose confirmed that these were UDP sugars. The use of the cold probe facilitated the assignment of carbon chemical shifts for the highly unstable UDP-arabinio-sugar as the 13C HSQC spectrum was obtained in a few hours (Fig. 3f). HMBC spectra (data not shown) were also obtained within a comparatively short time span using the cold probe and were pivotal in assigning the quaternary C-4 carbons for both II and IV.
UDP-α-d-GlcNAc (Fig. 4, line D, peak III). Coupling of these two enzymes resulted in loss of both enzymatic activities, resulting in a preparation that was identical to the starting material UDP-α-d-GlcNAc (data not shown), indicating an inhibitory protein-protein interaction. In contrast, no such effect was observed for the Pse enzymes. By uncoupling the two Bac reactions, large scale production of peak III was obtainable (Fig. 4, line D).

**NMR Analysis of HP0366, Cj1294, and Cj1121c Products**—Using one- and two-dimensional homo- and heteronuclear NMR experiments, the purified product of Cj1121c (III) was unambiguously identi-

| Compound | H-1 δ/ (ppm) | C-1 δ/ (ppm) | J(1,2)/Hz | H-2 δ/ (ppm) | C-2 δ/ (ppm) | J(2,3)/Hz | H-3 δ/ (ppm) | C-3 δ/ (ppm) | J(3,4)/Hz | H-4 δ/ (ppm) | C-4 δ/ (ppm) | J(4,5)/Hz | H-5 δ/ (ppm) | C-5 δ/ (ppm) | J(5,6)/Hz | H-6 δ/ (ppm) | C-6 δ/ (ppm) | Nac δ/ (ppm) | CH3 J/Hz |
|----------|--------------|--------------|----------|--------------|--------------|----------|--------------|--------------|----------|--------------|--------------|----------|--------------|--------------|----------|--------------|--------------|---------|---------|
| UDP-α-d-GlcNAc (I) | 5.51 | 3.99 | 3.81 | 3.55 | 3.92 | 3.82/3.86 | 2.08 |
| UDP-α-d-Xylo-sugar (II) | 5.45 | 4.10 | 3.82 | 94.9 | 53.1 | 72.3 | 4.12 | 1.24 | 2.07 |
| UDP-α-d-Bac (III) | 5.50 | 4.11 | 3.94 | 95.0 | 54.6 | 67.9 | 4.11 | 3.11 | 4.31 | 1.38 | 2.07 |
| UDP-β-L-Arabino-sugar (IV) | 5.55 | 4.24 | 3.92 | 94.1 | 53.5 | 70.0 | 4.06 | 1.37 | 2.07 |
| UDP-6-deoxy-β-L-AltNAc4N (V) | 5.67 | 4.20 | 4.30 | 94.1 | 52.8 | 65.2 | 4.34 | 4.23 | 1.43 | 18.8 | 2.05 |
| Ribose (R) | 5.97 | 4.37 | 4.36 | 4.28 | 4.18/4.23 |
| Uridine (U) | 5.97 | 7.95 | 167.2 | 152.6 | 103.4 | 142.4 |

**TABLE 3**

NMR chemical shifts δ (ppm) and coupling constants J (Hz) for metabolites of the bacillosamine and pseudaminic acid biosynthetic pathways

Carbon and proton chemical shifts were referenced to an internal acetone standard (δH, 2.225 ppm, δC, 31.07 ppm).

**FIGURE 3.** NMR spectroscopy of the reaction products of Cj1293 (II and IV) directly in aqueous reaction buffer (90% H2O). The reaction buffer, in addition to II and IV, contained 25 mM NaPO4, pH 7.2, 50 mM NaCl, 0.8 mM pyridoxal phosphate, and 8 mM glutamate. a, 1H NMR spectrum. b, selective one-dimensional TOCSY (90 ms) of IV H-1. c, one-dimensional NOESY (800 ms) of IV H-1. d, one-dimensional NOESY (800 ms) of IV H-6. e, 31P HSQC spectrum (1 h). f, 13C HSQC spectrum (8 h). For selective one-dimensional experiments, excited resonances are underlined. Residue II represents UDP-2-acetamido-2,6-dideoxy-α-d-xylo-4-hexulose, residue IV is UDP-2-acetamido-2,6-dideoxy-β-L-arabino-4-hexulose, and R is ribose.
Biosynthetic Enzymes of the Pse and Bac Pathways

FIGURE 4. Capillary electrophoresis analysis of the reaction products obtained after catalysis of UDP-α-D-GlcNAc by His6SFCj1120c (A), Cj1293His6c (B), HP0840His6c (C), His6SFCj1120c/His6Cj1121c (D), Cj1293His6c/His6Cj1124 (E), and HP0840His6c/His6HP0366 (F). Reactions were performed at 37 °C using 1 mM UDP-α-D-GlcNAc and ~0.3 mg ml⁻¹ final enzyme concentration for 3.5 h. Coupled reactions also contained 1 mM PLP and 10 mM L-Glu. Peak I, UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose; Peak II, UDP-2-acetamido-2,6-dideoxy-β-D-1-arabino-4-hexulose; Peak III, Pgl enzyme product; Peak V, flagellar glycosylation enzyme product; a.u., arbitrary units.

Unique Substrate Specificity of HP0366/Cj1294 and Cj1121c Aminotransferases—Further confirmation that the true product of HP0840 or Cj1293 is UDP-2-acetamido-2,6-dideoxy-β-L-arabinose-4-hexulose is the finding that HP0366 and Cj1294 do not utilize UDP-2-acetamido-2,6-dideoxy-α-L-altronic acid (27). Similar to results obtained with 49349 Cj1294 by Obhi and Creuzenet (29) 11168 His6Cj1294 exclusively uses the initial HP0840/Cj1293 product (Fig. 6A), which we have refined and confirmed here as UDP-2-acetamido-2,6-dideoxy-β-L-arabinose-4-hexulose. In contrast, His6Cj1121c exclusively uses UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose as substrate (Fig. 6B). Due to the instability of UDP-2-acetamido-2,6-dideoxy-β-L-arabinose-4-hexulose, its quantity modestly declined over time in this reaction but not to the significant extent to which UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose was consumed (Fig. 6B). In addition, neither His6HP0366 nor His6Cj1294 was able to utilize the product of His6SFCj1120c (data not shown), further confirming that their substrate is not UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose.

Kinetic Analysis of Cj1121c and Cj1294—The substrate preparation containing UDP-2-acetamido-2,6-dideoxy-β-L-arabinose-4-hexulose and UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose in the ratio of 1:3 was prepared by incubating Cj1293His6c with UDP-α-D-GlcNAc. This preparation was suitable for studying both Pse and Bac aminotransferases. To note, inhibition due to the complex nature of the substrate material did not appear to be a significant factor, as comparable kinetic values were obtained using Cj1293His6c filtrates containing a 6:1 ratio of the keto-sugars above, respectively. Both enzymes provided substrate-saturable velocities as determined by Michaelis-Menten kinetic analysis (Fig. 7). Apparent His6Cj1121c kinetic values of \( k_{\text{cat}} = 0.048 \pm 0.0075 \) mM and \( k_{\text{m}} = 144 \pm 9.5 \) min⁻¹ were calculated using Ednie-Hofste plots (Fig. 7A, inset). Similarly, apparent His6Cj1294 kinetic values of \( k_{\text{m}} = 0.052 \pm 0.0055 \) mM and \( k_{\text{cat}} = 4.0 \pm 0.26 \) min⁻¹ were obtained using Ednie-Hofste plots (Fig. 7B, inset).

DISCUSSION

We have unequivocally demonstrated that the Pse and 2,4-diacteamido-Bac pathways of C. jejuni and H. pylori are discrete, and we have defined the initial enzymatic steps of both, stemming from the universal substrate UDP-α-D-GlcNAc. As summarized in Fig. 8, the 2,4-diacteamido-Bac pathway starts with the conversion of UDP-α-D-GlcNAc to UDP-2-acetamido-2,6-dideoxy-α-D-xylo-4-hexulose by the C6 dehy-
Biosynthetic Enzymes of the Pse and Bac Pathways

FIGURE 5. NMR spectroscopy of the purified reaction product of HP0366 (V). a, $^1$H NMR spectrum (nt = 64). b, selective one-dimensional TOCSY (90 ms) of V H-1. c, one-dimensional TOCSY (90 ms) of V H-6. d, one-dimensional NOESY (800 ms) of V H-1. e, one-dimensional NOESY (800 ms) of V H-6. f, $^{13}$C HSQC spectrum ($J_{	ext{CH}} = 140$ Hz, 15 Hz). For selective one-dimensional experiments, excited resonances are underlined. Residue V represents UDP-2-acetamido-4-amino-2,6,3-dideoxy-$\beta$-altropyranosyl, and His ribose.

FIGURE 6. Aminotransferases from the Pse and Bac biosynthetic pathways utilize different substrates. Cj1294 uses exclusively UDP-2-acetamido-2,6-dideoxy-$\beta$-l-arabinopyranosyl-4-hexulose as substrate, whereas Cj1121c uses exclusively UDP-2-acetamido-2,6-dideoxy-$\alpha$-l-xylotripyranosyl-4-hexulose. His$_6$Cj1294 (A) or His$_6$Cj1121c (B) enzyme was incubated with Cj1293His$_6$ filter containing both substrates for the times indicated in the presence of 10 mM l-Glu and 1 mM PLP. Samples were then subjected to capillary electrophoresis analysis to obtain the relative peak areas for substrate(s) versus product. Squares, UDP-4-amino-4,6-dideoxy-$\beta$-l-altNAc product; diamonds, UDP-4-amino-4,6-dideoxy-$\alpha$-l-GlcNAc product; triangles, UDP-2-acetamido-2,6-dideoxy-$\alpha$-l-xylotripyranosyl-4-hexulose substrate; circles, UDP-2-acetamido-2,6-dideoxy-$\beta$-l-arabinopyranosyl-4-hexulose substrate.

pathway starts with the conversion of UDP-\(\alpha\)-D-GlcNAc to UDP-2-acetamido-2,6-dideoxy-\(\beta\)-l-arabinopyranosyl-4-hexulose by the C6 dehydratase/C5 epimerase Cj1293, or HP0840, followed by C4 aminotransfer of this 4-keto intermediate by Cj1294, or HP0366, to form UDP-4-amino-4,6-dideoxy-\(\beta\)-l-AltNAc (Fig. 8, steps I $\rightarrow$ IV $\rightarrow$ V). The final outcome of the C6 dehydratase reaction produces a C4 ketone (30), although the diol forms are depicted in Fig. 8 as the keto group rapidly converts to a diol in aqueous solution. Due to the flexibility of the C5 epimerized pyranose ring, the 4-keto and 4-amino derivatives can adopt various conformations, such as the \(^4\)C$_1$ and \(^4\)C$_4$ chair conformers depicted in Fig. 8. Because the formation of Pse has recently been shown to be catalyzed by a synthase that condenses phosphoenolpyruvate with 2,4-diacetamido-2,4,6-trideoxy-\(\beta\)-l-altropyranosyl (31), the only remaining steps leading to the formation of this hexose intermediate are, again, hydrolysis of the phosphate ester at C1 and N-acetylation at C4.

By using NMR to analyze the products of Cj1293 and HP0840 directly in non-purified aqueous reaction buffer, we have identified unequivocally UDP-2-acetamido-2,6-dideoxy-\(\beta\)-l-arabinopyranosyl-4-hexulose (IV) as a key intermediate of the pseudaminic acid pathway. In contrast, previous NMR analysis of the purified or lyophilized reaction products of these two enzymes indicated the presence of only UDP-2-acetamido-2,6-dideoxy-\(\alpha\)-D-xylo-4-hexulose (II) (data not shown). Although products II and IV were initially present in the aqueous sample, product IV was lost upon purification because of its extreme lability. The heightened sensitivity of the cold probe was also instrumental in characterizing the structure of IV because high quality $^1$H- and $^1$H-$^{13}$C-correlated NMR data could be obtained in comparatively short order. Intramolecular NOEs and $^{3}$J$_{HH}$ values measured for IV and V provided evidence indicating that the ring structures of these sugars are in dynamic equilibrium between the \(^4\)C$_1$ and \(^4\)C$_4$ conformations. These findings are in excellent agreement for a sugar with the altrose configuration because the characteristically small energy barrier separating the \(^4\)C$_1$ and \(^4\)C$_4$ conformations of altrose (0.8 to $\sim$1.5 kJ/mol) promote pseudorotation (27, 28). Because of its lability and flexible ring, the product of Cj1293 and HP0840 (IV) has been overlooked until very recently and is at the root of the uncertainty and controversy surrounding the bacillosamine.
and pseudaminic acid pathways in *C. jejuni* and *H. pylori* (21, 22, 29, 32, 33). In addition, recent enzymatic characterization of Cj1294 suggested that its reaction product (V) was UDP-4-amino-4,6-dideoxy-α-D-GalNAc (29). However, the NMR data reported by Obhi and Creuzenet (29) lack the J4,5 coupling constant critical for the galacto assignment.

Importantly, to our knowledge, this is the first in vitro functional characterization of the novel N-linked C. jejuni enzymes Cj1120c and Cj1121c of the novel N-linked pathway. Due to anticipated difficulties of expressing the full-length putative membrane protein Cj1120c, we characterized a soluble derivative consisting of residues 130–590, lacking the 4 putative transmembrane regions. This His6SFCj1120c derivative still retained activity, yet when incubated in a coupled reaction with His6Cj1121c it was unable to utilize UDP-α-D-GlcNAc. This observation points to an in vivo Cj1120c/Cj1121c complex, where its activity could be regulated through secondary interactions, possibly involving the Cj1120c transmembrane region.

HP0840, Cj1293, and Cj1120c belong to a family of enzymes essential for the production of surface-associated virulence factors, including
C6 dehydratase/C4 reductase that utilizes UDP-biosynthesis, had indicated that this enzyme was a membrane-bound ples. Elegant studies on WbpM, an enzyme essential for B-band LPS enzymes are in fact only C6 dehydratases that utilize UDP-2-acetamido-2,6-dideoxy-4-deoxy-pendent aminotransferase involved in the production of UDP-4-amino-4,6-dideoxy-β-D-glucosamine (UDP-QuiNAc) (23). In this study we have shown the products of Cj1120c and WbpM are in fact UDP-2-acetamido-2,6-dideoxy-β-D-arabinopyranoside (UDP-2-acetamido-2,6-dideoxy-β-L-arabinopyranoside; V, UDP-4-amino-4,6-dideoxy-β-L-AntNAc. The assignment of roman numerals to each compound is consistent with other label designations found throughout the text. Compounds II and IV are drawn as the hydrated forms of their respective keto-sugars.

Distinct biosynthetic pathways leading to the production of either Pse or Bac the initial steps stemming from the universal substrate UDP-α-D-GlcNAc. I, UDP-α-D-GlcNAc; II, UDP-2-acetamido-2,6-dideoxy-α-D-xylono-4-hexulose; III, UDP-4-amino-4,6-dideoxy-α-D-GlcNAc; IV, UDP-2-acetamido-2,6-dideoxy-β-D-arabinono-4-hexulose; V, UDP-4-amino-4,6-dideoxy-β-L-AntNAc. The assignment of roman numerals to each compound is consistent with other label designations found throughout the text. Compounds II and IV are drawn as the hydrated forms of their respective keto-sugars.

In conclusion, this work has demonstrated the inherent complexity of deoxy sugar biosynthetic pathways in bacteria and the difficulty in deciphering these pathways due to lability of certain biosynthetic intermediates. The importance of structural characterization of the biosynthetic precursors should not be underestimated in assigning enzymatic function to proteins that have been identified as functional homologs on the basis of sequence homology alone. As a consequence of the work presented here and to be consistent with the pre-established nomenclature for Cj1129 (33), we propose that the HP0840 (flaA1) gene be renamed pseB to clearly indicate its role in the Pse biosynthetic pathway. In addition, we propose that the aminotransferase genes Cj1294 and HP0366 be given the designation pseC to follow this same nomenclature and to indicate their role in the Pse biosynthetic pathway. Cj1290c and Cj1121c should retain their pgf and pgf assignment as it has been convincingly shown here that they are involved in the production of 2,4-diacetamido-Bac, a precursor used in assembling the Pgl glycan. Future work combining x-ray crystallographic data with the structural and biochemical data presented in this study will undoubtedly assist in elucidating the reaction mechanisms of these novel dehydratase/epimerases and aminotransferases, as well as in unraveling the structural context important for their substrate specificities.

Acknowledgments—We thank Melissa Schur (Institute for Biological Sciences, National Research Council, Ottawa, ON) for excellent CE technical assistance. We thank Drs. A. Matte and M. Cugler (Biotechnology Research Institute, National Research Council, Montreal, QC) for providing the modified pET15b vector pFO4.
REFERENCES

1. Butzler, J. P., and Skirrow, M. B. (1979) Clivi. Gastroenterol. 8, 737–765.
2. Dunn, B. E., Cohen, H., and Blaser, M. J. (1997) Clivi. Microbiol. Rev. 10, 720–741.
3. Parkhill, J., Wren, B. W., Mangulig, K., Ketley, J. M., Churcher, C., Basham, D., Chillingworth, T., Davies, R. M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A. V., Moule, S., Pallen, M. J., Penn, C. W., Quail, M. A., Rajandream, M. A., Rutherford, K. M., van Vliet, A. H., Whitehead, S., and Barrett, B. G. (2000) Nature 403, 665–668.
4. Fouts, D. E., Mongodin, E. F., Mandrell, R. E., Miller, W. G., Rasko, D. A., Ravel, J., Brinkac, L. M., Deboy, R. T., Parker, C. T., Daugherty, S. C., Dodson, R. J., Durkin, A. S., Madupu, R., Sullivan, S. A., Shetty, J. U., Ayodeji, M. A., Shvartsbeyn, A., Schatz, M. C., Badger, I. H., Fraser, C. M., and Nelson, K. E. (2005) PloS. Biol. 3, e15.
5. Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. F., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E. F., Peterson, S., Lofton, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzgerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M., and Venter, J. C. (1997) Nature 388, 539–547.

Biosynthetic Enzymes of the Pse and Bac Pathways

17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York.
18. Brisson, J. R., Sue, S. C., Wu, W. G., McManus, G., Nghia, P. T., and Uhrin, D. (2002) in NMR Spectroscopy of Glycoconjugates (Jimenez-Barbero, J., and Peters, T., eds) pp. 59–93, Wiley-VCH, Weinheim, Germany.
19. Uhrin, D., and Brisson, J. R. (2000) in NMR in Microbiology: Theory and Applications (Barotin, J. N. and Portais, J. C., eds) pp. 165–190, Horizon Scientific Press, Wymonden, UK.
20. Berces, A., Whitfield, D. M., and Nukada, T. (2001) Tetradecuron 57, 477–491.
21. Creuzenet, C., Schur, M. J., Li, J. W., Wakarchuk, W. W., and Lam, J. S. (2000) J. Biol. Chem. 275, 34873–34880.
22. Creuzenet, C. (2004) FEBS Lett. 559, 136–140.
23. Creuzenet, C., and Lam, J. S. (2001) Mol. Microbiol. 41, 1295–1310.
24. Kneidinger, B., O’Riordan, K., Li, J., Brisson, J. R., Lee, J. C., and Lam, J. S. (2003) J. Biol. Chem. 278, 3615–3627.
25. Maclean, L. L., Vinogradov, E., Crump, E. M., Perry, M. B., and Kay, W. W. (2001) Eur. J. Biochem. 268, 2710–2716.
26. Knirel, Y. A., Vinogradov, E. V., Shashkov, A. S., Wilkinson, S. G., Tahara, Y., Dmitriev, B. A., Kochetkov, N. K., Stanislasvsky, E. S., and Mashilova, G. M. (2006) Eur. J. Biochem. 265, 659–669.
27. Immel, S., Schmitt, G. E., and Lichtenthaler, F. W. (1999) in Proceedings of the Ninth International Cyclodextrin Symposium, Santiago de Compostela, Spain, May 31–June 3, 1998 (Torres-Labandeira, J. J., and Vila-Jato, J. L., eds) pp. 41–48, Kluwer Academic Publishers, Dordrecht, Netherlands.
28. Ionescu, A. R., Berces, A., Zgierski, M. Z., Whitfield D. M., and Nukada, T. (2005) J. Phys. Chem. A 109, 8096–8105.
29. Obhi, R. K., and Creuzenet, C. (2005) J. Biol. Chem. 280, 20902–20908.
30. Allard, S. T., Beis, K., Giraud, M., Hegeman, A. D., Gross, J. W., Wilmouth, R. C., Whitfield, C., Graninger, M., Messner, P., Allen, A. G., Maskell, D. J., and Naismith, J. H. (2002) Structure 10, 81–92.
31. Chou, W. K., Dick, S., Wakarchuk, W. W., and Tanner, M. E. (2005) J. Biol. Chem. 280, 35922–35928.
32. Merks-Jacobs, A., Obhi, R. K., Bethune, G., and Creuzenet, C. (2004) J. Bacteriol. 186, 2253–2265.
33. Chou, W. K., Kelly, J. F., Logan, S. M., Ewing, C. P., and Guerry, P. (2002) Mol. Microbiol. 46, 587–597.
34. Chou, W. K., Logan, S. M., Ewing, C. P., and Guerry, P. (2001) J. Biol. Chem. 276, 34862–34870.
35. Young, N. M., Brisson, J. R., Kelly, J., Watson, D. C., Tessier, L., Lanthier, P. H., Jarrell, H. C., Cadotte, N., St. Michael, F., Aberg, E., and Skirrow, M. B. (2002) J. Biol. Chem. 277, 42530–42539.
36. Wacker, M., Linton, D., Hitchen, P. G., Nita-Lazar, M., Haslam, S. M., North, S. J., Panico, M., Morris, H. R., Dell, A., Wren, B. W., and Aebi, M. (2002) Science 298, 1790–1793.
37. Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P., and Blaser, M. J. (1988) J. Infect. Dis. 157, 472–479.
38. Szymanski, C. M., Yao, R., Ewing, C. P., Trust, T. J., and Guerry, P. (1999) Mol. Microbiol. 32, 1023–1030.
39. Schirm, M., Soo, E. C., Aubry, A. J., Austin, J., Thibault, P., and Logan, S. M. (2003) Mol. Microbiol. 48, 1579–1592.