Phenotypic Variation in Molecular Mimicry between Helicobacter pylori Lipopolysaccharides and Human Gastric Epithelial Cell Surface Glycoforms

ACID-INDUCED PHASE VARIATION IN LEWISX AND LEWISY EXPRESSION BY H. PYLORI LIPOLYSACCHARIDES*

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The Gram-negative bacterium Helicobacter pylori is a prevalent pathogen of humans, and chronic infection of the gastric mucosa by the bacterium causes recurrent gastroduodenal inflammatory disease (1). H. pylori is a major cause of chronic gastritis and plays a pivotal role in the development of both gastric and duodenal ulcers (2–4). Moreover, persistent infection with this bacterium is associated with an increased risk for the development of gastric adenocarcinoma and primary lymphoma (5, 6). H. pylori is a chronic pathogen, and the mechanisms by which this bacterium is able to persist in the stomach and resist or evade destruction by the immune system is central to its pathogenesis (1, 7).

In part, survival of H. pylori in the stomach may be attributed to the development of specialized characteristics, including the capacity to withstand and adapt to exposure to gastric acidity. H. pylori colonizes the gastric mucosa layer where the pH gradient ranges from pH 2 on the luminal side to almost pH 7 on the epithelial cell surface (8). The helical shape and rapid motility of the bacterium facilitate its movement within viscus mucus, allowing the bacterium to escape extremely low pH (7, 9). Nevertheless, H. pylori cells must survive exposure to acidic pH during the early stages of gastric infection before colonization of the gastric mucus. Although the mucus layer provides a partial barrier to the acid contents of the stomach, H. pylori may encounter periodic exposure to low pH depending on the location of the bacterium in the gastric mucosa and host gastric physiology (9). In addition, H. pylori can alter the normal gastric physiology whereby acute H. pylori infection in humans is associated with transient hypochlorhydria, possibly facilitating enhanced intragastric survival early in infection, whereas chronic H. pylori infection leads to increased gastric acid secretion (10, 11). Below pH 4 survival of H. pylori is dependent on urease (12), an enzyme whose activity is essential for colonization (13), which liberates NH3 from urea that has been deduced to contribute to neutralization of gastric acidity (7). However, above pH 4 urease-independent mechanisms are involved in survival but remain to be elucidated fully.

Like the outer membrane of other Gram-negative bacteria, that of H. pylori contains lipopolysaccharide (LPS)† (14). Fresh

†The abbreviations used are: LPS, lipopolysaccharide; PS, polysaccharide; DQF-COSY, double-quantum filtered correlation spectroscopy; ELISA, enzyme-linked immunosorbent assay; MS, mass spectrometry; ESI MS, electrospray ionization MS; GLC, gas-liquid chromatography; DD-Hep, 2-glyceroph-phosphate-heptose; LD-Hep, 2-glucorono-2-phosphate; LA, lipid A; LacNAc, N-acetyllactosamine; LD, laser desorption; Lex, Lewisx (similarly for other Lewis antigens); OS, oligosaccharide;
clinical isolates of *H. pylori* produce high molecular mass smooth-form LPS, which consists of a polysaccharide (PS) O-chain, a core oligosaccharide (OS), and a lipid moiety, termed lipid A (15, 16). The first detailed structural analysis of *H. pylori* LPS showed that the PS region of the *H. pylori* type strain (NCTC 11637) was composed of an elongated, partially fucosylated N-acetyllactosamine (LacNAc) polysaccharide attached to the core OS and terminated at the non-reducing end by mono-, di-, or trimeric Lewis a (Le a)2 (17). Subsequent structural studies (18–25) and serological investigations (26–30) have shown that the O-chains of *H. pylori* strains express partially fucosylated, glucosylated, or galactosylated LacNAc chains of various lengths that may or may not be terminated at the nonreducing end by Le a and or Le b type 2 units, in mimicry of normal human cell-surface glyconjugates and of glycan antigens found in adenocarcinoma tumors (31, 32). In addition, certain *H. pylori* strains express fucosylated LacNAc chains terminated with Le a and Le b and blood group A antigenic determinants (22, 25, 33). The pathogenic relevance of Le a and Le b mimicry for *H. pylori* remains unclear but has been suggested to aid colonization by camouflaging the bacterium in the gastric mucosa and by aiding bacterial adhesion (14, 34–36), whereas in chronic infection this mimicry may induce antibodies contributing to the development of gastritis and influence the inflammatory response in the gastric mucosa (7, 14, 27, 29, 30).

Cultivation of *H. pylori* on solid agar media in vitro can result in a shift to production of low molecular mass rough-form LPS lacking O-chain expression and Le a/Le b mimicry (16, 17), but this can be reversed and production of smooth-form LPS stabilized when strains are grown in liquid media (16, 37). In addition to this variation, serological investigations suggest that phase variation (also called antigenic variation) in the type of Lewis antigen expressed on *H. pylori* can occur in vitro and in vivo (38, 39), but this requires verification by chemical studies because serological investigations of Le expression in *H. pylori* LPS can be misleading (22, 40). Moreover, compared with growth at neutral pH, growth of *H. pylori* at low pH on solid media has been reported to induce changes in colony morphology, cellular lipids, and virulence properties, possibly reflecting changes in bacterial wall characteristics in a low pH environment (41).

Despite these observed changes in total cellular lipids, no data are available on the influence of low pH on *H. pylori* LPS chemical structure or whether such conditions could induce phase variation in Le a and Le b expression. Because different Lewis antigens are expressed at different sites within the gastric mucosa (31, 42, 43), the ability of the bacterium to vary Le a and Le b antigen expression in response to environmental conditions such as pH could aid colonization at the different sites. In this paper, we investigated acid-induced changes in the structure and composition of LPS and cellular lipids. Bacteria were grown at pH 5 and 7 in a liquid medium, rather than on a solid medium, to avoid other changes such as smooth- to rough-form LPS shift, which may occur independently of pH (15, 16). Acid-induced changes in O-chain structure including phase variation in Le a and Le b expression were demonstrated independent of lipid A structure and cellular lipid composition. In addition to influencing the type of camouflage employed by *H. pylori*, such changes in the major glycolipid of the outer membrane would have an important influence on the properties of this membrane and would contribute to the adaptation of *H. pylori* to its ecological niche.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strain and Growth Conditions—** *H. pylori* strain 26695, whose complete genome sequence has been determined, was originally isolated from a patient with gastritis (40). This strain was grown routinely on blood agar under microaerobic conditions at 37 °C for 48 h as described previously (15, 37). Stock cultures were maintained at −70 °C in tryptophic soy broth containing 15% (v/v) glycerol (45). For induction of antigenic variation, *H. pylori* 26695 was grown in brain heart infusion containing 2% (v/v) fetal calf serum supplemented with 50 mM potassium phosphate buffer at pH 7 or in the same medium at pH 5 after adjustment of the pH by the addition of HCl (37, 41). Bacteria were harvested by centrifugation (5000 × g, 4 °C, 30 min) and washed twice if the pellets were used.

**Isolation and Degradation of Lipopolysaccharides—** After pretreatment of bacterial biomass with Pronase (Calbiochem), LPSs were extracted by the hot phenol-water technique (15). The water-soluble LPS preparations were purified by treatment with RNase A, DNase II, and proteinase K (Sigma) as described previously (15, 45) and subsequently by gel-permeation chromatography on a column of Bio-Gel P2 (1 m × 1 cm) eluted with the eluent (22). Only one carbohydrate-positive fraction (46) was obtained, which eluted in the high molecular mass range, consistent with previous observations (18, 22). These intact LPSs were used for chemical and serological analyses.

The LPSs were degraded with 0.1 M sodium acetate buffer, pH 4.2, at 100 °C for 4 h to cleave the acid-labile ketonic linkage between the core OS and lipid A. The water-insoluble lipid A was removed by centrifugation (5000 × g, 4 °C, 30 min), washed, and lyophilized separately. The supernatant was fractionated by gel-permeation chromatography on a column of Sephadex G-50 (70 × 2.6 cm; Amersham Biosciences, Inc.) using 0.05 M pyridinium acetate, pH 4.5, as eluent and monitored with a Waters differential refractometer. The resulting water-soluble carbohydrate-containing fractions (46) of O-chain and core OS preparations were lyophilized and redissolved in 0.1 M NaOH and subjected to hydrazinolysis (100 °C, 48 h) and N-acetylation with acetic anhydride/NaOH (47), and subsequently the product was purified by gel-permeation chromatography on columns of Sephadex G-50 (70 × 2.6 cm). Preparation of the lipid A backbone was accomplished by the degradative procedure described previously (45). Briefly, after treatment with 1 M HCl (100 °C, 30 min), lipid A was reduced with NaBH 4, and subjected to hydrazinolysis (100 °C, 48 h) and N-acetylation with acetic anhydride/NaOH (47), and subsequently the product was purified by gel-permeation chromatography on columns of Sephadex G-25 (50 × 2 cm) and TSK-HW40S (24 × 1 cm; Merck) (45).

**Electrophoretic and Serological Analyses—** For analysis of the macromolecular heterogeneity of *H. pylori* LPS by gel electrophoresis, proteinase K-treated whole-cell lysates were prepared as described (48). These lysates and isolated LPSs were analyzed by SDS-PAGE using a stacking gel of 5% (w/v) acrylamide and a separating gel of 15% (w/v) acrylamide containing 3.2% urea (15). After electrophoresis with a constant current of 35 mA for 1 h, the gels were fixed, and LPS was detected by silver staining (49). Alternatively, the fractionated LPS was electrophoresed onto nitrocellulose membranes by using the buffer system of Towbin et al. (50). Nitrocellulose membranes with transferred LPSs were probed with mouse IgM monoclonal antibodies against Le antigens anti-Le a (clone P12), anti-sialyl-Le a (clone CSLEX1), anti-Le b (clone F3), anti-Le a (clone T174), anti-Le b (clone T128) or H type I antigen (clone 17–206) (Signet Laboratories, Dedham, MA) or against blood group determinants anti-A, -B, or -AB (Immunocor, Norcross, GA) diluted 1:1000 as primary antibody and peroxidase-conjugated goat anti-mouse IgM (Sigma) diluted 1:1000 as the secondary antibody as described previously (29). Reactions in Western blots were visualized with the Bio-Rad pre-mixed enzyme substrate kit (2.5 ml of 4-chloro-1-naphtol in diethylene glycol, 25 ml of Tris-buffered saline, and 15 μl of H2O2) according to the manufacturer’s instructions.

Also, an enzyme-linked immunosorbent assay (ELISA) with bacterial whole-cell lysate was used as described previously (29) to examine the reaction of the anti-Le and blood group antibodies with *H. pylori* 26695 grown at pH 7 and 5. Protein concentrations of bacterial suspensions were determined using a commercial assay (Pierce). Subsequently, flat-bottomed microtiter plates were coated overnight with 100 μl of cell suspensions, with a protein concentration of 60 μg/ml, in 0.05 M NaHCO3 coating buffer, pH 9.6, and blocked with 3% (w/v) bovine

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**Phase Variation in H. pylori Lipopolysaccharides**

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5766

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*F*etn, 2-aminooethyl phosphate; Hep, heptose; OHC 16, 3-hydroxyhexadecanoic acid; OHC 18, 3-hydroxyoctadecanoic acid.

* Lewis a and Lewis b were originally defined as positional isomers of Lewis a and Lewis b blood group antigens, respectively, but are unrelated to phenotypes of the Lewis blood group system. To indicate their different origins, they will be referred to solely as Lewis a and Lewis b.
serum albumin at room temperature for 2 h. The ELISA assay was performed by the procedure of Wirth et al. (28). As described previously (29), the specificities of the antibodies in the assay were validated by their ability to bind the respective antigen from a panel of synthetic Le and blood group antigens (Isosep AB, Tullinge, Sweden and Dextra Laboratories Ltd, Reading, UK) and the LPSs of other H. pylori strains, H. pylori NCTC 11637, P466, and MO19, of known structure (17–19). The criterion that an absorbance value of <0.1 units was considered a negative result, whereas higher values were considered positive (28), was used in these whole-cell ELISA studies. All assays were repeated in triplicate.

Preparation, Identification, and Fractionation of Cellular Lipids—Lipids were extracted from bacterial homogenates using the method of Bligh and Dyer (51). Thin-layer chromatography (TLC) of total lipids was performed on silica gel 60 plates (Merek) using chloroform/methanol/water (75:22:3, by volume) as the solvent system. Detection was by charring with 1% (w/v) CeSO₄ in 10% (v/v) H₂SO₄ or by staining with the respective reagents: molybdate stain for phospholipids, ninhydrin for amino lipids, α-naphthol stain for glycolipids, and sulfonic acid/acetic acid reagent for sterols and sterol esters (52). The lipids L-α-phosphatidylethanolamine, L-α-lysophosphatidylethanolamine, L-α-phosphatidyl-DL-glycerol, cardio-lipin, phosphatidylcholine, and L-α-phosphatidyl-1-serine were obtained from Sigma and used as standards on TLC plates. The relative abundance of lipids was determined by scanning laser densitometry after charring of TLC plates (53).

Total lipid extracts were subjected to lipid anion-exchange chromatography on 1 ml (100 mg) Superlean LC-NH₄⁺ solid phase extraction columns (Supelco, Bellefonte, PA) as described previously (41, 54). Fractions were sequentially eluted with the solvents: chloroform/2-propanol (2:1, v/v), diethyl ether/acetic acid (98:2, v/v), acetonitrile/2-propanol (2:1, v/v), methanol, and 2-propanol/3 m methanolic HCI (4:1, v/v) to yield five fractions, A to E (41). The two predominant phospholipids, phosphatidylethanolamine and lysophosphatidylethanolamine, were purified from fraction D on silica gel 60 (40–60 μm, Merek) using chloroform/methanol/water (75:22:3, by volume) as the eluent, and the appropriate fractions were collected and dried under N₂. The identities of these fractions were confirmed by GLC-MS.

Sugar Composition and Methylation Linkage Analyses—Sugar composition analysis of LPSs was performed by the alditol acetate method (55). Hydrolysis of glycosidic bonds was achieved with 2 m trifluoroacetic acid at 120 °C for 2 h and followed by reduction with NaBD₄, and acetylation with acetic anhydride (100 °C, 1 h). The respective de-derivatives were identified by gas-liquid chromatography (GLC) using a Hewlett-Packard 5880 chromatograph (Avondale, PA) equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm) and a temperature program of 160 °C (1 min) to 260 °C at 3 °C/min and by GLC-mass spectrometry (MS) on a Hewlett-Packard 5890 chromatograph equipped with a NERMAG R10 microprobe mass analyzer (LAMMA 500, Leybold AG, Cologne, Germany) with acetonitrile as the mobile phase at a flow rate of 10 μl/min; the samples were dissolved in aqueous 50% acetonitrile and 10 μl was directed via a syringe pump into the electrospray source. LD MS was carried out with a laser microprobe mass analyzer (LAMMA 500, Leybold AG, Cologne, Germany). Free dephosphorylated lipid A was mixed with either NaI or CsI to obtain fragments as well as molecular ions in the positive ion mode as described (45, 65).

NMR Spectroscopy—Samples were exchanged twice with D₂O. ¹H NMR spectra of D₂O solutions were run on a JEOL EX-270 instrument at 75 °C or on a Varian Inova 600 instrument at 25 °C. Two-dimensional ¹H–¹H COSY and ¹H–¹³C heteronuclear single-quantum coherence experiments were performed on the Inova 600 instrument. Chemical shifts are reported in ppm using internal sodium 3-trimethylsilylpropanoate-d₄ (δH 0.00) or internal dioxane (δH 67.40). For lipid analysis, ³¹P NMR spectra were recorded on a Varian 500 Unity instrument at 35 °C, spectra were broadband ¹H-decoupled, and samples were referenced to an 80% (w/v) solution of phosphoric acid (0.00 ppm) as an external standard (54, 55).

Electrospray ionization (ESI) and Laser Desorption (LD) Mass Spectrometry—ESI MS was performed in the negative mode using a VG Quattro triple quadrupole mass spectrometer (Micromass, Altrincham, Cheshire, UK) with acetonitrile as the mobile phase at a flow rate of 10 μl/min; the samples were dissolved in aqueous 50% acetonitrile at a concentration of ~50 pmol/μl and 10 μl was directed via a syringe pump into the electrospray source. LD MS was carried out with a laser microprobe mass analyzer (LAMMA 500, Leybold AG, Cologne, Germany). Free dephosphorylated lipid A was mixed with either NaI or CsI to obtain fragments as well as molecular ions in the positive ion mode as described (45, 65).

RESULTS

SDS-PAGE and Serological Analyses—When analyzed in SDS-PAGE with silver staining (Fig. 1), isolated LPSs from H. pylori 26695 biomass grown at pH 5 and 7, LPS1 and LPS2, respectively, showed profiles characteristic of slow migrating, high molecular mass LPS with PS O-chains as reported previously (15, 16). Like the purified preparations of LPS1 and LPS2, proteinase K-treated whole-cell lysates of biomass grown under the differing pH conditions gave identical electrophoretic profiles, indicating that the LPS extraction and purification procedure did not affect the macromolecular nature of the bacterial lipopolysaccharide. These experiments showed that the O-PS portion of LPS1 expressed both Le⁻ and Le⁰, whereas LPS2 expressed only Le⁰ (Fig. 2). No reaction of these LPSs with monoclonal antibodies against other Le antigens (anti-Le⁻, -Le⁰, or -H type 1) or against blood group determinants (anti-A⁻, -B, or -AB) was observed. In the case of chemical modification or selection of a particular LPS molecular species...
Phase Variation in *H. pylori* Lipopolysaccharides

**Fig. 2. Immunoblots of LPSs from *H. pylori* 26695 grown at pH 5 (LPS1) and pH 7 (LPS2) with anti-Le" and anti-Le* mononclonal antibodies.** Lane A, pH 5; lane B, pH 7. LPS1 and LPS2 were prepared as described in Fig. 1 and under “Experimental Procedures.” SDS-PAGE was performed using a stacking gel of 5% (w/v) acrylamide and a separating gel of 15% (w/v) acrylamide containing 3.2% urea under a constant current of 35 mA for 1 h. The fractionated LPSs were electroblotted onto nitrocellulose membranes (50), and the transferred LPSs were probed with anti-Le" and anti-Le* mouse IgM monoclonal antibodies diluted 1:1000 as primary antibody and peroxidase-conjugated goat anti-mouse IgM diluted 1:1000 as the secondary antibody as described under “Experimental Procedures.” Positive reactions can be seen between LPS1 and anti-Le" and Le* monoclonal antibodies, whereas only anti-Le* monoclonal antibody reacted with LPS2.

**Fig. 3. Whole-cell ELISA of *H. pylori* 26695 grown at pH 5 (LPS1) and pH 7 (LPS2) with anti-Le*, anti-Le*, anti-Le antigen, and anti-blood group mononclonal antibodies.** The ELISA with bacterial whole cells was used as described previously (28,29). These data indicate that *H. pylori* 26695, when grown at pH 5 expresses Le* and Le* antigens, whereas at pH 7, only Le* antigen is expressed. Only absorbance values >0.1 units were considered positive (28).

during extraction, the same panel of antibodies was tested for reaction in an ELISA with whole cells of *H. pylori* grown at pH 5 and 7, respectively (Fig. 3). Using the criteria previously established for the ELISA (28), the observed expression of Le* and Le* at pH 5 and only Le* at pH 7 was consistent with the LPS1 and LPS2 immunoblotting results, respectively.

**Structural Analyses of the LPS Core**—Sugar analysis of PS1, including determination of the absolute configurations of the monosaccharides, revealed L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose in the molar ratios 0.4:1.4:1.0 as the main components. In addition, ribose, glucose, DD-Hep, and LD-Hep were identified as core constituents of *H. pylori* LPS as described above.

**Methylation analysis** of PS1 (Table I) resulted in identification of partially methylated alditol acetates derived from the major components: terminal Fuc, terminal and 3-substituted Gal, 4-substituted GlcNAc, and 3,4- and 4,6-disubstituted GlcNAc. These data suggested that PS1 is branched with two different lateral sugar residues (Gal and Fuc) and two different GlcNAc residues as branch point residues. Comparison with the methylation analysis data for LPS1 (Table I) showed that no terminal fucose was cleaved during mild acid degradation. In addition, a number of minor partially methylated alditol acetates were detected. Most of them were derived from the core region of LPS, as determined by comparison with the methylation analysis data of OS1 (Table I). However, two minor products originated from PS1. One was derived from 3,4,6-trisubstituted GlcNAc and showed that a minor portion of GlcNAc residues carried two side chains. The other was from 2-substituted Gal, which is located in the terminal nonreducing LacNAc unit of PS1 (see Structure 3).

In the 1H NMR spectrum of the PS1, a signal from PETn was observed at δ 3.28 (t, J 5.0 Hz, CH2N) with a cross-peak to δ 4.13 (CH2OP) in the two-dimensional 1H,1H DQF-COSY spectrum. In the two-dimensional 1H,13C heteronuclear single-quantum coherence spectrum of the PS1, a correlation was for 1 with a calculated molecular mass of 1785.53 (in contrast, a peak at m/z 870.2 would be observed for the phosphate monoester derivative that would have a calculated molecular mass of 1742.46 Da). The 3-deoxy-D-manno-octulosonic acid (Kdo) residue at the reducing end of 1 and the other core OS was found to exist in an anhydroform (anhKdo).

Heterogeneity of OS1 was revealed by ESI MS (Fig. 4) and found to be associated with the lack of one or two hexose residues (peaks of doubly charged ions, [M-2H]2–, at m/z 810.53 and 730.01, respectively) and attachment of up to three additional hexose residues (m/z 972.7, 1053.91, and 1134.55) or two hexose and one pentose (ribose) residue (m/z 1120.01). The major compound in the mixture corresponded to 1 with one additional hexose residue attached. As indicated by the methylation analysis data (Table I), this and other additional monosaccharides may substitute the terminal Glc residue at position 3 or 6 or position 2 of the 7-substituted DD-Hep residue in 1 (see Refs. 18–20). The exact sites of their attachment, as well as their sequence and anomeric configurations remain to be determined.

The disaccharide fragment α-L-Fucp-(1→3)-β-D-GlcNAc present in OS1 is also a constituent of the PS O-chain (see Structures 3 and 4). In the methylation analysis of OS1, the Fuc residue appeared as 3-substituted (Table I), whereas this residue is terminal, as followed from other data for OS1, including ESI MS data (Fig. 4). This phenomenon has been observed previously (18) and has been suggested to originate from incomplete methylation.

Similar analyses of OS2, the core OS derived from *H. pylori* LPS2, showed essentially the same structure but with a lower degree of chain elongation such that the contribution of compounds with more than one additional hexose residue was negligible. The major compound in the mixture was the Glc-lacking oligosaccharide 2 (see Structures 1 and 2). Therefore, except for the degree of glucosylation, there is no significant difference between the core regions of the two LPSs.

**Elucidation of the Structure of the PS O-Chain from *H. pylori* LPS1 (PS1)—Sugar analysis of PS1, including determination of the absolute configurations of the monosaccharides, revealed L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose in the molar ratios 0.4:1.4:1.0 as the main components. In addition, ribose, glucose, DD-Hep, and LD-Hep were identified as core constituents of *H. pylori* LPS as described above.
observed from a $^1$H NMR signal at $\delta$ 3.28 to a $^{13}$C NMR signal at $\delta$ 41.9, further supporting the presence of a PEtn group.

Treatment of PS1 with aqueous 48% hydrofluoric acid resulted in a modified polysaccharide (PS1M) that eluted from Sephadex G-50 with a similar elution volume as PS1 soon after the void volume of the column. Sugar analysis of PS1M yielded terminal and undegradable core-related oligosaccharides after the reaction, which is deduced from the presence of a poly($\beta$-GlcNAC) chain and showed that the lateral Gal and Fuc residues are attached at position 6 of GlcNAc and GalNAc, respectively.

Table I

| Sugar                  | Relative retention time | Detector response |
|-----------------------|-------------------------|-------------------|
|                       |                         | OS1 | PS1 | LPS1 | PS1M | PS2 |
| 2,3,4-Me$_3$-Fuc$^a$  | 1.00                    |     | 5   | 3    | <1   | 8   |
| 3,4-Me$_2$-Rib       | 1.02                    | <1  | 4   | 3    | <1   | 5   |
| 2,4,6-Me$_3$-Fuc      | 1.34                    |     | 11  | <1   | 4    | 2   |
| 2,3,4,6-Me$_3$-Glc    | 1.51                    |     | 3   | 14   | 11   | 27  |
| 2,4,6-Me$_3$-Gal      | 1.86                    | 7   | 2   | 4    | 5    |
| 2,3,6-Me$_3$-Gal      | 1.89                    | 13  | 4   | 7    | 6    |
| 2,3,6-Me$_3$-Glc      | 1.92                    | 2   | <1  | <1   |
| 2,4,6-Me$_3$-Gal      | 1.97                    | 4   | 31$^b$ | 23$^b$ | 45 | 35 |
| 3,4,6-Me$_3$-Gal      | 1.97                    | 4$^b$ | 3$^b$ |
| 2,3,4-Me$_3$-Glc      | 2.20                    | 5   | <1  | <1   |
| 2,3,4,6,7-Me$_4$-Hep  | 2.13                    | <1  | 1   |
| 3,4,6,7-Me$_4$-Hep    | 2.85                    | 4   | <1  | 2    | 2    |
| 2,3,4,6,7-Me$_4$-Hep  | 2.92                    | 6   | <1  | 2    | 2    |
| 2,3,4,6,7-Me$_4$-Hep  | 3.16                    | 12  | 3   | 5    | 3    |
| 2,3,4-Me$_3$-dd-Hep   | 3.46                    | 10  | 3   | 2    |
| 2,3,6-Me$_3$-GlcN     | 3.60                    | <1  | 7   | 5    | 14   | 17 |
| 2,4,6-Me$_3$-GlcN     | 3.89                    | 8   | <1  | 4    | 2    |
| 2,3,4-Me$_3$-GlcN$^a$ | 3.96                    | 4   |     |
| 2,6,6-Me$_3$-GlcN     | 4.14                    | <1  | 6   | 3    | 8    |
| 2,3,6-Me$_3$-GlcN     | 4.45                    | <1  | 10  | 6    | 1    |
| 2-Me-GlcN             | 4.95                    | 1   | <1  |

$^a$ The value is low owing to a putative loss of the volatile derivative.
$^b$ The values are taken from mass fragmentographic analysis from a 1:10 nonseparable mixture of 3,4,6-Me$_3$Gal and 2,4,6-Me$_3$Gal.
$^c$ From lipid A of LPS.

\[
\text{R-}(1\rightarrow4)-\alpha-\text{Gal} \\
\text{1} \downarrow \\
\text{PEtn} \\
\text{7} \\
\beta-\text{GlcNAC-}(1\rightarrow7)-\text{D-}\alpha-\text{D-Hep-(1\rightarrow2)-D-}\alpha-\text{D-Hep-(1\rightarrow2)-L-}\alpha-\text{D-Hep-(1\rightarrow3)-L-}\alpha-\text{D-Hep-(1\rightarrow5)-anhKdo} \\
\text{3} \\
\uparrow \\
\text{1} \\
\alpha-\text{L-Fuc}
\]

1 $R = \alpha-\text{Glc}; 2 R = H$

Structures 1 and 2

The $^1$H NMR spectrum of PS1M contained, among other things, signals for three anomeric protons at $\delta$ 4.47 ($\beta$-Gal H1), split to two close doublets, $J_{1,2} = 7.5$ Hz for each, 4.74 ($\beta$-GlcNAC H1, $J_{1,2} = 8$ Hz), and 5.02 ($\alpha$-Gal H1, $J_{1,2} = 2.5$ Hz; the coupling, lower than expected, is probably due to second order effects). The ratios of the intensities of the anomeric signals were 1:1:0.45, respectively, which fitted well with the methylation analysis data. Therefore, the $^1$H NMR data confirmed the presence of a poly($\beta$-GlcNAC) chain and showed that the lateral Gal residue is $\alpha$-linked.

The $^1$H NMR spectrum of PS1 indicated a higher degree of structural heterogeneity due to nonstoichiometric substitution with two lateral monosaccharides, Gal and Fuc. The major signals for anomeric protons belonged to $\alpha$-Gal and $\alpha$-Fuc (both at $\delta$ 5.04, $J_{1,2} \sim 3$ Hz). 

The $^1$H, $^1$H DQF-COSY and $^1$H, $^{13}$C heteronuclear single-quantum
coherence experiments. Also, the chemical shifts of the C5 signals of the Fuc groups were observed at $\delta \sim 67.5$ ppm, corroborating the $\alpha$-anomeric configuration. Furthermore, the $^1$H NMR spectrum of PS1 contained signals for N-acetyl groups of GlcNAc ($\delta$ 2.02) and CH$_3$-C groups (H6) of Fuc ($\delta$ 1.14–1.26) with the ratio of intensities 1:0.35. In total, there were H6 signals for five Fuc residues, two of which appeared as separate doublets at $\delta$ 1.23 and 1.26 and three others as three superposed doublets at $\delta$ 1.14 ($J_{5,6} \sim 6$ Hz for all H6 signals, Fig. 5A). The two-dimensional DQF-COSY spectrum (Fig. 6) showed Fuc H6/H5 cross-peaks at $\delta$ 1.14/4.35, 1.14/4.81, 1.23/4.87, and 1.26/4.25. The first cross-peak was from the Fuc residue, which is adjacent to the LPS core region, as deduced from the DQF-COSY spectrum of OS1, where only one Fuc H6/H5 cross-peak was observed, and at the same coordinates (compare also the one-dimensional $^1$H NMR data, Fig. 5, B and C). The cross-peak at $\delta$ 1.26/4.25 was assigned to the Fuc group substituting...
position 2 of β-Gal in the terminal nonreducing LacNAc unit (compare e.g. with δ 4.22 for Fuc-H-5 in 2-[α-fucopyranosyl]lactose (66)). The cross-peak at δ 1.23/4.87, having the same intensity, originated from the Fuc residue of the Fuc-(1→3)-GlcNAc unit in a terminal Le y tetrasaccharide (24). The last cross-peak at δ 1.17 was from the Fuc residue of the Fuc-(1→3)-GlcNAc unit in a terminal Le x trisaccharide. The signals at δ 1.14 originate from Fuc in LacNAc units of the main chain and Fuc adjacent to the LPS core region.

Therefore, two Fuc residues are present at the nonreducing LacNAc unit of PS1, where they form a Le y unit (structural unit 3A), two more are attached to two interior LacNAc units (structural units 3C and 3E), and the fifth Fuc residue is attached to the unit that is adjacent to the LPS core. The structure of PS1 may be described by formula 3, in which the exact distribution of various interior structural units B-E along the PS chain is unknown. Based on the ratios of the GlcNAc derivatives revealed in methylation analysis (Table I) and on the Fuc and GlcNAc methyl group signal intensities in the 1H NMR spectrum, the average degree of polymerization in PS1 was estimated as 12–13 LacNAc units.

**Elucidation of the Structure of the PS O-chain from H. pylori LPS2 (PS2)—**Sugar analysis showed that the content of Gal in PS2 was significantly lower than in PS1 (the ratios of Fuc:Gal:GlcN were 0.35:0.95:1). Methylation analysis revealed the absence of 4,6-disubstituted GlcNAc and the presence of only a small amount of terminal Gal, whereas the content of the other PS constituents (terminal Fuc, 3-substituted Gal, 4-substituted GlcNAc, and 3,4-disubstituted GlcNAc) indicated a close similarity between PS2 and PS1 (Table I). As deduced from the ratio of methylated GlcNAc derivatives, the degree of fucosylation in PS2 was 30%. No 2-substituted Gal was detected, but instead, terminal Gal was present (Table I), and therefore, unlike PS1, PS2 has no Le y antigenic determinant.

The 1H NMR spectrum of PS2 was similar to that of PS1 but lacked the H1 signal for α-Gal. Also, the signals for H6 of Fuc displayed a different pattern; there was a doublet at δ 1.17 and superposition of three doublets at δ ~ 1.14 (J$_{5,6}$ ~ 6 Hz for all H6 signals, Fig. 5B). As expected, signals at δ 1.23 and 1.26, which belonged to the Fuc residues from the terminal Le y tetrasaccharide in PS1 (Fig. 6), were absent from the spectrum of PS2. The ratio of the integral intensities of the signals for methyl groups of Fuc (δ 1.14–1.17) and GlcNAc (δ 2.03) was 0.32:1, again indicating incomplete fucosylation of LacNAc units.

The two-dimensional DQF-COSY spectrum of PS2 showed, among other things, Fuc H6/H5 cross-peaks at δ 1.23/4.33, 1.14/4.81, and 1.17/4.83. The two first cross-peaks were also present in the spectrum of PS1 (Fig. 6) and belonged to Fuc residues attached to the core region and to the interior LacNAc units, respectively (see Structure 3). The last cross-peak was absent from the spectrum of PS1 and was assigned to the Fuc residue located at the terminal nonreducing LacNAc unit of PS2, where it forms an Le x unit (24). As in PS1, there are only two Fuc residues attached to interior LacNAc units, and the average degree of polymerization of PS2 was estimated as 12 LacNAc units.

These data suggest that PS2 has structure 4, which contains terminal fucosylated LacNAc (structural unit 4A) and both non-fucosylated (structural unit 4B) and fucosylated LacNAc (structural unit 4C). This structure differs from structure 3 of PS1 only in the absence of substitution by lateral α-Gal residues and the second α-Fuc residue from the nonreducing LacNAc unit, producing a terminal Le y trisaccharide (structural unit 4A) rather than Le y tetrasaccharide.

**Structural Analysis of Lipid A—**Compositional analysis of free lipid A preparations from LPS1 and LPS2 (LA1 and LA2) revealed, beside fatty acids, a similar composition of GlcN and phosphate in the molar ratio 2:1.4, with trace amounts of ethanolamine. The fatty acids present in both lipid A preparations were dodecanoic (C$_{12}$), tetradecanoic (C$_{14}$), hexadecanoic (C$_{16}$), octadecanoic (C$_{18}$), 3-hydroxyhexadecanoic (OHC$_{16}$), and 3-hydroxyoctadecanoic (OHC$_{18}$) acids in the approximate molar ratios 0.5:0.4:0.1:1.0:1.3:2.0, respectively.

Treatment of these lipid A preparations with 0.1 M HCl (100 °C, 30 min) liberated glycosidic phosphate (1 eq), and subsequent reduction with NaBH$_4$ yielded products containing...
D-GlcN and D-glucosaminitol (1.0:0.9) with residual phosphate content (0.4 eq), attributable to ester-bound phosphate as reported previously (45). Subjecting these products to the chemical degradation pathway for lipid A backbone analysis developed earlier (45, 47) yielded N-acetylated disaccharides of GlcNAcβ1–6GlcNAc-ol with identical properties in high pressure liquid chromatography, identical 1H NMR spectra, and after permethylation, identical GLC-MS mass spectra as authentic standards (47). Analysis by 31P NMR spectroscopy of both LA1 and LA2 revealed a signal (2.96 ppm) for a glycosidic phosphate and ethanolamine-phosphate groups, respectively, since acidic treatment (0.1 M HCl, 100 °C, 30 min) led to their liberation and loss of both signals. A further but weaker signal corresponding to an ester-bound phosphomonoester (4.52 ppm), which was acidstable, was attributed to a 4′-phosphate group (45). Collectively, these data showed that the backbones of LA1 and LA2 were identical 1,4-bisphosphorylated β(1,6)-linked GlcN disaccharides.

To study the distribution of fatty acids on the lipid A backbone, dephosphorylated LA1 was subjected to LD MS after cationization by a NaI admixture and analyzed in the positive-ion mode (Table II). Analysis of dephosphorylated LA1 by LD MS after admixture of CsI gave a similar fragmentation pattern and showed the presence of nonstoichiometric amounts of amide-bound C16O18 on the nonreducing GlcN unit of the lipid A backbone (data not shown). Collectively, the data showed that the reducing GlcN of the backbone carries ester-bound 3-hydroxyoctadecanoic and ester-linked (OHC16), whereas the nonreducing GlcN can carry amide-bound C18O18 and ester-bound C12O16 or C14O16 (Fig. 7), identical to the fatty acid distribution reported previously in H. pylori NCTC 11637 (45). However, heterogeneity in the acylation pattern in lipid A from smooth-form LPS of H. pylori has been observed whereby tetraacyl lipid A predominates, but hexaacyl lipid A is also present (45, 68). Therefore, to resolve this issue, dephosphorylated LA1 was subjected to silica gel chromatography (47), and one major and a second minor fraction were obtained. Analysis by LD MS of these fractions showed the predominance of tetraacyl lipid A (without ester-bound fatty acids bound to the nonreducing GlcN unit), whereas analysis of the minor fraction showed the hexaacyl distribution (Table II). Identical data were obtained for LA2. Collectively, these results show no comparative difference in the structure of LA1 and LA2.

**Composition of Cellular Lipids**—The content of total lipids in H. pylori 26695 grown at pH 5 and 7 was similar (7.2 and 7.4% w/w, respectively). The neutral phospholipid fraction from both contained phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine at 91.6, 8.3, ~0.05, and ~0.05% (w/w), respectively. In contrast to a previous report (41), there was no elevation in phosphatidylserine content when this H. pylori strain was grown at pH 5. Furthermore, the fatty acid composition of phosphatidylethanolamine of H. pylori 26695 grown at pH 7 was C16 (29.5%), C16 (13.7%), C16 (3.6%), C17 (0.3%), C16 (4.6%), C18:1 (4.6%), C18:2 (0.2%), C19 (1.1%), C19 (53.4%) but did not differ significantly from that of growth at pH 5.

### TABLE II

**Assignment of peaks in the LD mass spectra of dephosphorylated lipid A derived from H. pylori 26695 LPS1 (LA1)**

| m/z | Cleavage process | Structure |
|-----|------------------|-----------|
| 1914 | [M − C12 + C4 + Na]+ | C12−C4+Na+ |
| 1866 | [M + Na+] | C18+Na+ |
| 1858 | [M − C12 + C4 + Na]+ | C12−C4+Na+ |
| 1704 A | [M − C12 + H + Na]+ | C12+H+Na+ |
| 1614 B | [M − OHC16 + H + Na]+ | OHC16+H+Na+ |
| 1449 C | [M − C12 − OHC16 + H2O + Na]+ | C12−OHC16+H2O+Na+ |
| 1432 D | [M − C12 − OHC16 + H2O + Na]+ | C12−OHC16+H2O+Na+ |
| 1230 G | [M1 + 42 + N 1]+ | M1+42+Na |
| 1196 C, E | [M − OHC16 − H − C12 − OHC16 + H + Na]+ | OHC16+H−C12−OHC16+H+Na+ |
| 1188 | [M + H]+ | M+H+ |
| 1183 C, F | [M − C18 − H − C12 − OHC16 + H + Na]+ | C18−C12−OHC16+H+Na+ |
| 1108 A, H | [M1 + 102 − C12 + H + Na]+ | M1+102−C12+H+Na+ |
| 1048 A, G | [M1 + 42 − C12 + H + Na]+ | M1+42−C12+H+Na+ |
| 1006 A | [M1 − C12 + H + Na]+ | M1−C12+H+Na+ |
| 853 C, H | [M1 + 102 − C12 − OHC16 + H + Na]+ | M1+102−C12−OHC16+H+Na+ |
| 793 C, G | [M1 + 42 − C12 − OHC16 + H + Na]+ | M1+42−C12−OHC16+H+Na+ |
| 751 C | [M1 − C12 − OHC16 + H + Na]+ | M1−C12−OHC16+H+Na+ |
| 739 C, M1 | [M1 + Na]+ | M1+Na+ |
| 585 C, F, H | [M1 + 102 − C12 + H + C12 − OHC16 + H + Na]+ | M1+102−C12+H+C12−OHC16+H+Na+ |
| 527 C, F, G | [M1 + 42 + C12 + H + C12 − OHC16 + H + Na]+ | M1+42+C12+H+C12−OHC16+H+Na+ |
| 465 C, F | [M − C12 + H + C12 − OHC16 + H + Na]+ | C12−C12−OHC16+H+Na+ |
| 456 C | [M − OHC16 + H + Na]+ | OHC16+H+Na+ |

* Peak that can be derived from tetraacyl lipid A as well as from cleavage of acyl chains from hexaacyl lipid.

**FIG. 7.** Interpretation of the fragmentation pattern of dephosphorylated lipid A derived from LPS1 (LA1). The numbers in circles refer to the number of carbon atoms in acyl chains, and the letters indicate the designated cleavage process. Refer to Table II for details of the formation of positive ions.

**DISCUSSION**

The present investigation on the chemical composition of LPSs from H. pylori grown at different pH values has added new insight into the expression of Lea and Leb mimicry by this bacterium, particularly phase variation. When grown in liquid
medium at pH 7, the O-chain of *H. pylori* 26695 (PS2) consisted of a LacNac polysaccharide that was glycosylated with α-L-Fuc at O-3 of the majority of GlcNAc residues forming Leα units, including chain termination by a Leα unit. However, growth in liquid medium at pH 5 resulted in production of a more complex O-chain (PS1) whose backbone of LacNac units was partially glycosylated with α-L-Fuc, thus forming Leα, whereas the majority of the nonfucosylated GlcNAc residues were substituted at O-6 by α-D-Gal residues, and the chain was terminated by a Leα unit. Thus, the nonreducing termini differ in the two O-chains as well as in glycosylation of the internal LacNac units. In contrast, detailed chemical analysis of the core and lipid A components of LPS and analysis of cellular lipids did not show significant differences between *H. pylori* 26695 grown at pH 5 and 7. Type 2 series, Leα, Leγ, and sialyl-Leα, and in cellular lipid composition that are associated with loss of the medium in vitro show significant differences between units. In contrast, detailed chemical analysis of the core and O-chains as well as in glycosylation of the internal LacNac units. Consequently, detailed chemical analysis of the core and lipid A components of LPS and analysis of cellular lipids did not show significant differences between *H. pylori* 26695 grown at pH 5 and 7. Type 2 series, Leα, Leγ, and sialyl-Leα, and in contrast, detailed chemical analysis of the core and O-chains as well as in glycosylation of the internal LacNac units. Nevertheless, in serological surveys of strains from different geographical regions, Leα and Leγ predominate, expressed by >80% of strains (26–30). Consistent with the results of this study, previous structural studies on other *H. pylori* strains have reported that Leα, when present in smooth-form LPS, occurs as the terminal unit on the O-chain, whereas Leγ occurs terminally and as an internal unit in the O-chain (17–20, 22–25, 33). Furthermore, the fatty acid composition of phosphatidylethanolamine, a predominant amount of lysophosphatidylethanolamine and, elevated phosphatidylserine at the lower pH, whereas phosphatidylethanolamine predominated, and phosphatidylerine was a minor constituent at neutral pH. Also, the fatty acid composition of phosphatidylethanolamine differed at pH 5 and 7. The investigators suggest that the differing colony morphology and virulence properties of the strain grown at the lower pH reflected changes in the polarity of the bacterial cell wall due to a changed lipid composition because of a bacterial response to the acidic environment. In contrast, in the present study no elevation in content of lysophosphatidylethanolamine and phosphatidylserine was observed for *H. pylori* 26695 grown on solid media at pH 5 and 7 found a much reduced amount of phosphatidylethanolamine, a predominant amount of lysophosphatidylethanolamine, and elevated phosphatidylserine at the lower pH, whereas phosphatidylethanolamine predominated, and phosphatidylerine was a minor constituent at neutral pH. Also, the fatty acid composition of phosphatidylethanolamine differed at pH 5 and 7. The investigators suggest that the differing colony morphology and virulence properties of the strain grown at the lower pH reflected changes in the polarity of the bacterial cell wall due to a changed lipid composition because of a bacterial response to the acidic environment. In contrast, in the present study no elevation in content of lysophosphatidylethanolamine and phosphatidylserine was observed for *H. pylori* 26695 grown in a liquid medium at pH 5 nor did the fatty acid composition of phosphatidylethanolamine differ at pH 5 and 7 in solid media. Likewise, no significant differences were found between lipid A components of bacteria grown in liquid media at pH 5 and 7. The observed changes in lipid composition on solid media by Bukholm et al. (41) may be dependent on the physical nature of the growth medium rather than pH. Consistent with this, prolonged subculturing and growth of *H. pylori* on solid medium in vitro, independent of pH, can induce changes in cellular lipid composition that are associated with loss of the O-chain and a shift from smooth- to rough-form LPS (15, 16). In contrast, fresh clinical isolates of *H. pylori* produce smooth-form LPS, indicating that high rather than low molecular mass LPS is produced in vivo (15). However, growth of *H. pylori* in liquid media in vitro stabilizes production of smooth-form LPS containing a PS O-chain (16, 37), and thus, these conditions were used in the present study when examining the influence of environmental pH.

Further demonstrating the importance of the physical nature of the growth medium in these investigations, *H. pylori* 26695, when grown on solid medium, was shown previously to produce a low molecular mass semi-rough LPS carrying a single Le unit, mostly a Leα unit, but alternatively, type 1 and type 2 linear B blood group and Leα attached to the core OS, whereas when grown in liquid medium, the strain produced smooth-form LPS (25). The O-chain of the latter was composed of a polyfucosylated LacNac chain terminated with a Leα unit, corresponding to PS1 in this study. Although no significant differences were observed between the core OS of bacteria grown in liquid media at pH 5 and 7 in the present study, these cores differed from that of *H. pylori* 26695 when grown on solid media at neutral pH (25) by the predominant absence of a glucan chain substituting α-Gal on the fourth heptose (DD-Hep) residue.

The genome of *H. pylori* 26695 and that of another strain, J99, contain two copies of α(1,3)-fucosyltransferase (*HpfucT*) genes that are required for expression of Leα and Leγ but differ in the number of a seven-amino acid sequence repeat, YDDLVN (44, 69). DNA motifs near the 5’-end of these genes (HP0379 and HP0651) at two distinct polynucleotide repeats have been deduced to indicate regulation through slipped-strand repair (44). No putative gene for α(1,2)-fucosyltransferase (*HpfucT2*), which is required for Leγ synthesis, was initially identified in the 26695 genome (44), but a truncated gene (HP0094) with a C14 tract was found, and in silico insertion of a C-G pair yielded a full-length protein with strong homology to α(1,2)-fucosyltransferase (70). Moreover, sequence analysis has shown that the *HpfucT2* gene contains a mutually complementary sequence (poly-C and TTA repeats) that provides a possibility of frequent shifting into and out of coding frame by a polymerase slippage mechanism (71). Phase variation in expression of Leα has been attributed to changes in the lengths of poly-C tracts in the *HpfucT* genes (38), but the lengths of these tracts has not been found to be predictive of the phenotype (72). On the other hand, variable expression of Leγ by *H. pylori* strains has been proposed to occur at the combined levels of replication slippage (mutation), transcription, and translation of the *fucT2* gene (71). Despite these deductions as to the putative molecular mechanisms involved in variable Leα and Leγ expression by *H. pylori*, the resultant phenotypes of phase variants were not established previously in detailed structural studies. Furthermore, the environmental trigger inducing variable Leα and Leγ expression was not identified.

The present study addresses such issues by showing that relative pH can influence expression of Leα and Leγ, particularly at the termini of the O-chains, but also that pH influences glycosylation, including substitution at O-6 by α-D-Gal residues of the internal LacNac units of these chains. Consistent with these structural findings as well as the serological and electrophoretic investigations in this study, McGowan et al. (73) found qualitative differences in LPS electrophoretic profiles when *H. pylori* 60190 was grown at pH 5 compared with pH 7. Using subtractive RNA hybridization they identified an acid-inducible gene in this strain whose protein was highly homologous to that of *WbgJ* of enteric bacteria that is considered involved in the conversion of GDP-D-mannose to GDP-D-fucose. Moreover, a corresponding gene (HP0045) occurs in the 26695 genome, which had previously been designated a *nolK* homologue based on 44% identity with *NolK*, an inducible nodulating protein of *Azorhizobium caulinodans* (44). Thus, mechanisms affecting fucose availability in addition to fucosyltransferase activity are present in *H. pylori* 26695 that can influence Leα and Leγ expression.

4 A. P. Moran, unpublished results.
Common to the genomes of both sequenced strains, *H. pylori* 26695 and J99 are seven open reading frames encoding putative glycosyltransferases (HP0159/JHP147, HP0208/JHP194, HP0619/JHP563, HP0679/JHP620, HP0805/JHP741, HP0826/JHP765, and HP1105/JHP1031) that have been implicated in LPS core synthesis, but in addition there are three strain-specific open reading frames (JHP562, JHP820, and JHP1032) in strain J99 and one (HP1578) in strain 26692, reflecting differences in the core OS of the two strains (25, 69). Three genes in the 26695 genome (HP0159, HP0208, and HP1416) are homologues of the α(1,2)-glucosyltransferase (rfaD) found in enteric bacteria (69, 74), and although it has been debated whether some of these genes encode β(1,4)-galactosyltransferase and/or β(1,3)-N-acetylglucosaminyltransferase functions needed for type 2 chain synthesis (70), α(1,2)-substituted Glc can occur in the core of *H. pylori* 26695 under certain growth conditions (25). Also, homologues of galactosyltransferases from *Klebsiella pneumoniae* have been suggested to be involved in O-chain synthesis in strain 26695 (74). However, rather than involvement in addition to β(1,4)-substituted Gal in type 2 chains, these may be required for addition at O-6 of nonfucosylated GlcNAc residues by α-α-Gal residues, as observed in PS1.

Several enteric bacteria are known to vary gene expression in response to acid stress (75). However, there has been no evidence that exposure to acidic pH alters expression of their LPS-associated genes. On the other hand, compared with gonococcal cells grown at pH 8.2, those grown at pH 5.8 produce LPS with an altered electrophoretic profile, indicative of pH regulation (76), but this has not been characterized structurally. In *Rizobium leguminosarum*, LPS is modified in response to several environmental stresses, including low pH (77). Loss of a plasmid carrying acid tolerance genes from *H. pylori* 26695 under certain growth conditions (76), but this has not been characterized structurally, in type 2 chains, these may be required for addition on O-6 of nonfucosylated GlcNAc residues by α-α-Gal residues, as observed in PS1.

*Phase Variation in H. pylori Lipopolysaccharides*

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Phenotypic Variation in Molecular Mimicry between *Helicobacter pylori* Lipopolysaccharides and Human Gastric Epithelial Cell Surface Glycoforms: ACID-INDUCED PHASE VARIATION IN LEWISX AND LEWISY EXPRESSION BY H. PYLORI LIPOLYSACCHARIDES

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