Phenotypic Modulation of Gonococcal Lipooligosaccharide in Acidic and Alkaline Culture

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Neisseria gonorrhoeae infects a diverse array of niches in its human host, which expose the organism to dramatic variations in pH. We examined growth and lipooligosaccharide expression of two gonococcal strains in liquid and solid cultures under acidic, neutral, and alkaline conditions. Growth rates in broth were similar under the three conditions, and the pH remained fairly constant throughout the growth cycle. Altered lipooligosaccharide expression at the different pHs was noted in both plate- and broth-grown organisms.

Neisseria gonorrhoeae will colonize almost any mucosal surface in its human host, including the urethra, vagina, rectum, conjunctiva, and pharynx (1). In addition, viable organisms are often located within neutrophils and epithelial cells (1, 3). These diverse environments expose the gonococcus to dramatic variations in oxygen tension, available nutrients, reactive oxygen intermediates, and pH.

A number of studies have been published concerning the response of *N. gonorrhoeae* to thermal stress (14), nutrient stress (9), and anaerobic stress (2). A particularly relevant environmental stress for *N. gonorrhoeae* is pH fluctuation, because this organism can be cultured from host tissues exhibiting remarkable differences in pH. For example, the average vaginal pH in infected and uninfected women is 5.5 (range, 4.1 to 7.4), and the average pH of male urethral exudates is 7.3 (range, 6.2 to 8.4) (5).

We investigated growth and lipooligosaccharide (LOS) expression of two gonococcal strains ranging from pH 5.8 to 8.2. Because liquid and solid cultures likely reflect different in vivo conditions, growth and LOS expression were evaluated with both types of culture. Strains FA19 (serum resistant, protein 1A bearing [11, 12]) and F62 (serum sensitive, protein 1B bearing [11, 12]) were maintained on gonococcal typing agar (pH 7.0). Nonpiliated, non-Opa-bearing organisms were selected (13). Medium buffer was modified for pH experiments with a final concentration of 30 mM MES [2-(*N*-morpholino)ethanesulfonic acid; pK_a , 6.1; useful pH range, 5.5 to 6.7 (SigmaUltra)] for pH 5.8 experiments and 30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pK_a , 7.5; useful pH range, 6.8 to 8.2 [SigmaUltra]) for pH 7.2 and 8.2 experiments. Final pH adjustment was made by addition of NaOH to media at 37°C. The pH of all preparations was verified after sterilization.

pH 7.0-grown organisms were used to inoculate pH 5.8, 7.2, and 8.2 broth cultures, and growth curves of shaken cultures were obtained. Cell densities over time under the three conditions were remarkably similar (Fig. 1). In contrast, Morse and Hebel (8) found the generation time of gonococcal strain CS-7 at pH 6.0 was almost twice that of pH 7.5-grown CS-7. One obvious difference in their methods was the use of HEPES for broth cultures prepared at pHs 6 through 8.

pH was monitored during growth in broth (Table 1). Under

these conditions, the gonococcus does not appear to be able to significantly change the pH of its environment. Interestingly, both acidic and alkaline cultures shifted slightly toward neutrality, but even in late stationary phase, they remained acidic or alkaline. Similar results were obtained when these experiments were repeated on different days.

FIG. 1. Growth curves of gonococcal strains FA19 (A) and F62 (B) at pH 5.8 (□), 7.2 (◆), and 8.2 (■). Abs, absorbance.

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TABLE 1. pHs of broth cultures throughout growth cycle

Time (h)	FA19			F62		
	pH 5.8	pH 7.2	pH 8.2	pH 5.8	pH 7.2	pH 8.2
0	5.75 ^a	7.23	8.21	5.80	7.37	8.20
1	6.11	7.34	8.15	6.17	7.43	8.14
2	6.10	7.32	8.06	6.13	7.39	8.02
3	6.10	7.26	7.96	6.14	7.31	7.90
4	6.05	7.18	7.83	6.11	7.21	7.75
5	6.08	7.19	7.83	6.13	7.22	7.72
9	6.17	7.22	7.72	6.10	7.28	7.71

^a Data are pHs at indicated times.

Aliquots from broth cultures were removed at the late log phase, washed, and solubilized, and protein concentrations were determined in Coomassie brilliant blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. LOS phenotypes were first characterized in standard size silver-stained gels of whole-cell lysates (Fig. 2A and B). Samples were then digested with proteinase K (PK) (6), separated in 14% mini-gels (Bio-Rad Mini Protean II) containing 2.67 M urea, and stained with silver nitrate (Fig. 2C and D). In both whole-cell lysates and PK digests of cells cultured at the three pHs, strain F62 but not strain FA19 exhibited altered LOS. Of note is the appearance of the two LOS bands in PK digests of strain F62 (Fig. 2D). Certain gonococcal strains exhibit an acquired form of serum resistance due to the covalent transfer of host sialic acid to the galactose in the terminal lactoside of LOS (10). pH 7.2-grown F62 cells expressed the 4.5-kDa band, which accepts sialic acid (7), in greater quantity than pH 5.8- and 8.2-grown F62 cells. pH 5.8- and 7.2-grown F62 cells consistently expressed the 4.9-kDa band, which does not accept

FIG. 2. Silver (17.5%)-stained SDS-PAGE gels of broth-grown FA19 (A) and F62 (B) cells and their PK digests in 14% mini-gels with 2.67 M urea (C and D, respectively). Numbers across the top are pHs. MW, molecular mass markers (kilodaltons). Arrowheads denote LOS alterations (see the text for details).

FIG. 3. Silver (17.5%)-stained SDS-PAGE gels of plate-grown FA19 (A) and F62 (B) cells and their PK digests in 14% mini-gels with 2.67 M urea (C and D, respectively). Numbers across the top are pHs. MW, molecular mass markers (kilodaltons). Large arrowheads denote LOS alterations; small arrowheads denote protein alterations (see the text for details).

sialic acid (7), in greater quantity than pH 8.2-grown F62 cells. The 4.9-kDa LOS component contains the binding site for bactericidal immunoglobulin M in normal human serum (7, 10), so gonococci growing at alkaline pH might be less susceptible to killing by normal human serum.

In contrast to broth, growth on agar at pH 5.8 appeared stressful, particularly for strain FA19. After 16 h of incubation, lawns of bacteria were obtained at pH 7.2 and 8.2, while relatively few colonies had formed on pH 5.8 agar. The LOS phenotype of 16-h agar cultures was examined by SDS-PAGE as described for broth cultures. The LOS of FA19 cells cultured on plates at pH 5.8 was markedly less abundant than the LOS of pH 7.2- and 8.2-grown FA19 cells (Fig. 3A and C, large arrowheads). LOS bands of pH 7.2-cultured F62 cells consistently appeared altered with respect to the LOS of pH 5.8- and 8.2-grown F62 cells (Fig. 3B and D). As in broth, the 4.9-kDa band in pH 8.2-cultured F62 cells was less abundant than in pH 5.8- and 7.2-cultured F62 cells (Fig. 3D, large arrowhead). For both strains, growth on agar at the different pHs resulted in the induction and repression of several proteins (Fig. 3A and B, small arrowheads). Of particular interest was the increased expression of several high-molecular-weight proteins, possibly heat shock proteins, at pH 5.8.

In the course of producing disease, *N. gonorrhoeae* must experience anaerobiosis and exposure to acidic and alkaline conditions. Frangipane and Rest (4) recently reported that anaerobically grown F62 cells (solid culture) expressed the 4.5-kDa acceptor for sialic acid in greater quantity than aerobically grown F62 cells and that aerobically grown F62 cells (solid culture) expressed the 4.9-kDa band in greater quantity than anaerobically grown F62 cells. In addition, they found

that broth-grown aerobic gonococci were much more readily converted to serum resistance by sialic acid than were agar-grown gonococci.

These studies have important implications for the growth of *N. gonorrhoeae* in vivo, where oxygen is limiting and pH is site dependent.

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