Inhibition of *Neisseria gonorrhoeae* by *Lactobacillus* Species That Are Commonly Isolated from the Female Genital Tract

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Epidemiological studies suggest H2O2-producing lactobacilli protect women against gonorrhea. Here we demonstrate that *Lactobacillus crispatus* and *Lactobacillus jensenii*, the most common lactobacilli in the female genital tract, inhibit gonococci in both acidic and neutral pH conditions. Inhibition was neutralized by bovine catalase, suggesting that H2O2 is the primary mediator of inhibition.

*Neisseria gonorrhoeae* has a major impact on health worldwide, with the highest morbidity and mortality occurring in females. The most common site of gonococcal infection in females of reproductive age is the endocervix (9). A variety of host factors may contribute to the success or failure of *N. gonorrhoeae* to infect this site, including the types of commensal flora that inhabit the lower genital tract. Lactobacilli, the most common facultatively anaerobic bacteria of the vagina (13) and endocervix (7), play an important role in maintaining a normal vaginal ecosystem through the production of organic acids, bacteriocins, and hydrogen peroxide, all of which may protect against pathogens (13). Among the many microbes that inhibit *N. gonorrhoeae* in vitro (2, 8, 15, 16), lactobacilli are of particular interest due to reported associations between a reduced risk of gonorrhea and colonization by lactobacilli (1, 10, 15).

*Lactobacillus crispatus* and *Lactobacillus jensenii* are the predominant *Lactobacillus* spp. in the female lower genital tract. *Lactobacillus acidophilus* and *Lactobacillus gasseri* are also frequently isolated (1, 4, 6, 14). Inhibition of *N. gonorrhoeae* in vitro has been reported only for *L. acidophilus* (20), however, and for unidentified H2O2-producing clinical isolates of lactobacilli (15). The capacity of the predominant *Lactobacillus* spp. of the genital tract to inhibit *N. gonorrhoeae* is therefore unclear. Here we tested H2O2-producing strains of *L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. acidophilus* for the capacity to inhibit two gonococcal laboratory strains (MS11 and FA1090) that are infectious in male volunteers (3, 18) and four clinical isolates of *N. gonorrhoeae* (Table 1) using a modified version of the agar overlay technique of Saigh et al. (15). Briefly, saline suspensions containing ca. 10⁶ CFU of lactobacilli harvested from lactobacillus-MRS agar plates per ml were prepared. Fifty-microliter samples of the suspensions were inoculated onto heart infusion agar (HIA) that was adjusted to the desired pH (range, 5.8 to 7.6) prior to autoclaving. After 20 to 24 h of incubation, 7.5 ml of GC agar were poured onto the HIA plates and allowed to solidify. Suspensions (100 μl) containing ca. 10⁶ CFU of the *Neisseria* species or *Escherichia coli* strains to be tested (target organisms) were spread onto the agar overlay and incubated for 20 to 24 h. The presence of a zone of growth inhibition around the target strain was considered positive for inhibition. For all experiments, the number of CFU in the lactobacilli and target cell suspensions was confirmed by standard serial dilution and culture. Growth of lactobacilli on HIA did not appreciably change the pH of the agar as determined by the use of pH indicators (data not shown). All media were purchased from Difco Laboratories (Detroit, Mich.). All incubations were at 37°C in 5% CO₂.

All four lactobacillus strains inhibited all gonococcal strains tested at low pH; only *L. jensenii* and *L. crispatus* inhibited *N. gonorrhoeae* at neutral pH. None of the lactobacilli inhibited *E. coli*, and only *L. jensenii* inhibited *Neisseria cinerea*, a commensal organism of the respiratory and genital tracts (Table 2). Serial dilution of the lactobacillus suspensions before inoculating the base agar resulted in visibly fewer lactobacilli within the inoculated region. On the basis of this semiquantitative evaluation of the number of lactobacilli present during the assay, *L. jensenii* consistently demonstrated higher levels of inhibition against *N. gonorrhoeae* than the other three lactobacillus strains (Fig. 1). Inoculation of the overlay agar with >10⁶ CFU of *N. gonorrhoeae* significantly reduced the zones of inhibition and reproducibility of the assay (D. J. Kuch and A. E. Jerse, unpublished observations).

The primary mediator of inhibition in all four strains appeared to be H2O2 based on the ability to neutralize inhibition by incorporating bovine catalase (Warthington Biochemicals, Lakewood, N.J.) into the overlay medium. Inhibition of *N. gonorrhoeae* by *L. crispatus* and *L. jensenii* when cultured at pH 7.0 was neutralized by 5 U of bovine catalase per ml. At an acidic pH, a 10-fold-higher concentration of catalase was required to neutralize inhibition by *L. jensenii*, and 100-fold more catalase was required to neutralize inhibition by *L. acidophilus* and *L. crispatus* (Table 3). This result was reproducible, although it appears to be inconsistent with the large inhibitory zones produced by *L. jensenii* compared to those produced by *L. crispatus* (Fig. 1). In general, more catalase was required to neutralize the inhibition by all lactobacilli as the

pH of the base agar decreased (pH 7.6, 7.0, 6.6, 6.3, and 5.8) (data not shown). This observation may be explained by increased production of H₂O₂ at low pH or increased stability of H₂O₂ at low pH (5).

These data support the hypothesis that commensal lactobacilli in the lower genital tract reduce the risk of gonococcal infection in women through the production of H₂O₂. This observation may be explained by increased production of H₂O₂ by lactobacilli at low pH or in the presence of bovine catalase (data not shown). This observation may be explained by increased production of H₂O₂ by lactobacilli at low pH or in the presence of bovine catalase (data not shown).

The relationship between culture pH and the relative inhibitory potential of each Lactobacillus species tested is intriguing in light of the cyclical change in pH of the female lower genital tract. The average pH values of vaginal and cervical mucus are as follows: pH 5.8, pH 7.0, pH 7.4, pH 8.0, and pH 8.5.

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during the proliferative stage of the menstrual cycle are 4.6 (range, 3.3 to 7.4) and 6.8 (range, 5.5 to 8), respectively. A lower pH occurs in the luteal stage, with an average vaginal pH of 4.4 (range, 3.6 to 6.0) and endocervical pH of 6.1 (range, 5.1 to 8.4) (17). On the basis of these data, one might hypothesize that the capacity of commensal lactobacilli to protect women against gonorrhea may depend on both the species and stage of the menstrual cycle. The capacity of L. jensenii or L. crispatus to inhibit N. gonorrhoeae at both low and neutral pHs suggests that these strains may protect against gonorrhea more effectively than the other species tested.

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REFERENCES


