Inhibition of *Candida albicans* by *Lactobacillus acidophilus*

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ABSTRACT

Candida albicans grew at pH 4.6 or above in nutrient broth containing 5% glucose but was retarded at pH 7.7 by filtrates of *Lactobacillus acidophilus* grown in casitone broth. Vaginal implantation of nonfermented acidophilus milk, yogurt, or low-fat milk for preventing recurrence of monilia vaginitis subsequent to treatment with Nystatin was studied with 30 women. Reinfections within 3 mo according to product received were: no milk product, 3; yogurt, 1; nonfermented acidophilus milk, 1; and low-fat milk, 0.

INTRODUCTION

Yeast infections of the vaginal canal (monilia vaginitis) are a distressing and frequently recurring problem of women. Such infections are caused primarily by *Candida albicans* (6) and apparently occur when the environmental balance in the vagina is disturbed. From puberty to menopause lactobacilli, primarily *Lactobacillus acidophilus* (11, 17), exist in large numbers in the vagina (1, 7) and contribute to maintenance of acid pH through fermentation of carbohydrates particularly glycogen (6, 11, 14, 17). If the lactobacilli are suppressed, yeast or various bacteria increase in numbers and cause irritation and inflammation. Broad-spectrum antibiotics (e.g., tetracycline), which can kill the indigenous bacterial flora, have been implicated (5, 8). Yeast infections usually are treated with antimycotic agents (Nystatin), but recurrence of infection is common (10).

Lactobacilli have been implanted in the vagina subsequent to treatment with Nystatin in attempts to prevent recurrence of infection. Butler and Beakly (1) isolated a lactobacillus from one woman, grew large populations of the organism, and treated patients with 7 million lactobacilli per patient; Ostrzenski (10) treated patients with a lyophilized culture of *L. acidophilus* suspended in cocoa butter, and Gunston and Fairbrother (4) treated patients with yogurt. Each study gave inconclusive evidence that lactobacilli were beneficial.

This work was to study inhibition of *C. albicans* by *L. acidophilus.* Several reports show that lactobacilli produce metabolites that inhibit various bacteria (3, 9, 12, 13, 16), but few investigators have included fungi in their studies. Guillot (3) reported that an inhibitor produced in casein broth by *L. acidophilus* inhibited *C. albicans.* We also conducted a clinical study to get data on the value of applying commercial products containing lactobacilli for preventing recurrence of vaginal yeast infections.

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MATERIALS AND METHODS

Organisms

Organisms were *Lactobacillus acidophilus* strain B (2), used extensively in preparing commercial nonfermented acidophilus milk, and *Candida albicans.* The latter, isolated from a patient who had monilia vaginitis, conformed with characteristics for *C. albicans* (15).

Media

Casitone broth was an aqueous solution of 1.5% Bacto-casitone sterilized by filtration. Where indicated, biotin (10^{-6} g/ml) was added. Bacto-nutrient broth containing 5% glucose was sterilized by heating at 121 C for 15 min.

Growth Measurements

Growth of *C. albicans* was followed by periodic measuring of optical density (OD) with a Klett-Summerson photoelectric colorimeter with filter no. 66. Cell mass was determined from a standard curve relating OD to dry weight. An OD 1.0 represented .59 μg (dry weight)/ml for *C. albicans.*

RESULTS

Inhibition of *C. albicans*

Prior to testing *L. acidophilus* for inhibition of *C. albicans*, the yeast was tested for growth in nutrient broth containing 5% glucose adjusted to pH's 2.8, 3.8, 4.6, 5.5, and 6.8. It grew best at pH 5.5 and 6.8. Growth at pH 4.6 was less, and growth at pH 3.8 and 2.8 was poor. Subsequently, we tested *L. acidophilus* for production of inhibitors in casitone broth, a medium similar to that used by Guillot (3). *Lactobacillus acidophilus* was grown in skim milk, transferred (1%) to casitone broth, and grown at 37 C for 24 h. The bacteria were removed by centrifugation, the supernatant was sterilized by filtration, and part of the sterile supernatant was autoclaved for 10 min at 121 C to inactivate any heat labile inhibitors (e.g., hydrogen peroxide). Fresh casitone broth, autoclaved supernatant, and unheated supernatant each received biotin (10^{-6} g/ml) and adjustment to pH 7.7 to stimulate *C. albicans* (3). The media then were dispensed (10 ml/flask; 4 flasks/medium) into Nephelo culture flasks (Bellco Glass Co., Vineland, NJ), inoculated with *C. albicans* (1% of a culture grown 24 h in casitone broth), and incubated statically at 37 C. (Growth of *C. albicans* was negligible in one experiment in which the media were adjusted to pH 4.2). Results are in Figure 1.

Candida albicans grew slower, and the maximal population was slightly less in filtrates of *L. acidophilus* than in casitone broth. With autoclaved filtrate, growth of *C. albicans* was improved over that in unheated filtrate. The experiment was repeated with a smaller inoculum of *C. albicans* (ca. 2000 cells/ml). Results were similar to those in Figure 1.

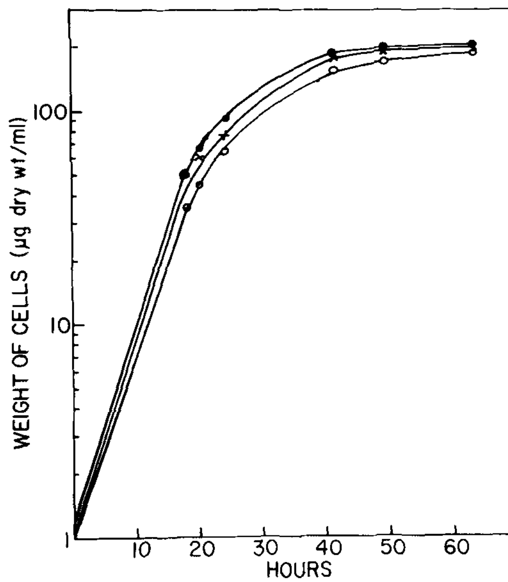

Figure 1. Growth of *Candida albicans* in filter-sterilized casitone broth (●) and in autoclaved (x) or filter-sterilized (○) casitone broth in which *Lactobacillus acidophilus* had been grown for 24 h at 37 C.

Clinical Study

Women infected with *C. albicans* were approached for participation in the research through the UCD Student Health Center and the Davis Free Clinic. Each volunteer was given a 10-day supply of Nystatin by her physician and randomly assigned to an experimental group for receiving no milk product or one of the following purchased locally and kept available for distribution: nonfermented acidophilus milk (made with the strain of *L. acidophilus* used above), yogurt, or low-fat milk. Each received oral and written instructions and was given a data sheet, a product, an applicator, and pH test paper (range, pH 3.0 to 5.5). Each volunteer was instructed to determine her vaginal pH once a day (at bedtime; not soon after urination) for 2 wk, twice per wk for 2 subsequent wk, once per wk for 2 additional wk, and once after 12 wk. Ten milliliters of the assigned milk product was to be implanted into the vagina immediately after each pH determination. An Emko applicator (The Emko Co., St. Louis, MO) was used for yogurt, and a Fleet Vet bottle (C. B. Fleet Co., Lynchburg, VA) was used for the other milk products. Each participant was phoned every 2 wk to remind her to pick up a fresh milk product, and each was phoned again after 3 mo to find whether there had been recurrence of the infection.

Thirty women completed a 3-mo study. There were no significant differences between the vaginal pH's reported by the groups. Most pH's were 4.0 to 4.5 throughout the study. There were occasional reports of pH 5.0; and, for the no-milk-product group, a few reports of pH 3.0 or 3.5. Reinfections according to product received were: no milk product, 3; yogurt, 1; nonfermented acidophilus milk, 1; and low-fat milk, 0.

DISCUSSION

Considerable evidence indicates that lactobacilli normally are in the vagina and may be important in suppressing growth of *C. albicans* (5, 8). The culture of *C. albicans* we studied grew well in nutrient broth containing 5% glucose at pH 4.6, within the pH range of the normal vagina (1), and it seems unlikely that production of lactic acid by lactobacilli can explain fully their beneficial role. It is probable that they suppress yeast in part by producing other inhibitors, and it is possible that lactobacilli growing naturally in the vagina are more inhibitory than laboratory cultures whose beneficial properties might have been diminished through serial transfer. Our laboratory data and that of Guillot (3) support this possibility by suggesting that *L. acidophilus* can produce metabolites that inhibit *C. albicans.*

Studies have shown inconclusively that addition of lactobacilli to the vagina can assist in preventing recurrence of monilia vaginitis (1, 4, 10). Our data also are limited, and we did not use lactobacilli freshly isolated from the vagina. Nevertheless, we found low-fat milk as inhibitory to yeast reinfections as nonfermented acidophilus milk or yogurt. Possibly lactose, which is a substrate for lactobacilli and not for *C. albicans* (15), stimulated growth of indigenous lactobacilli. Possibly any influence application of milk products has on control of monilia vaginitis can be explained as selective stimulation of indigenous or applied lactobacilli by lactose and concomitant increase in production of inhibitors.

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