JOURNAL OF CLINICAL MICROBIOLOGY, May 1992, p. 1323–1326
0095-1137/92/051323-04$02.00/0

Vol. 30, No. 5

Defined Medium Simulating Genital Tract Secretions for Growth of Vaginal Microflora

ALI M. GESHNIZGANI AND ANDREW B. ONDERDONK*

Departments of Pathology and Medicine, Channing Laboratory, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Received 21 November 1991/Accepted 8 February 1992

A chemically defined medium that simulates female genital tract secretions was developed for the growth of the vaginal microflora. Qualitative and quantitative studies of the growth of predominant components of the vaginal microflora indicated that all vaginal isolates tested were able to grow in this defined medium.

The bacterial populations residing within the human vaginal vault represent a complex ecosystem to which a wide array of host-derived substances also contribute. The microflora of the female genital tract has been extensively studied by means of advanced microbiological techniques (1, 11), as have the composition and characteristics of vaginal secretions at various stages of the menstrual cycle (6, 7, 9, 10, 15, 16). Disturbances of the vaginal ecosystem that lead to changes of the resident microflora may result in both "unexplained" vaginitis and documented genital tract infections (14). This suggests that the mechanisms that control bacterial populations in this environment are complex. Clearly the health of the female genital tract is at least partially dependent upon the maintenance of a balance among the various bacterial species making up the microbial flora. Genital tract secretions may play an important role in determining the interactions of the resident microbial populations with the host.

The fluid material that can be collected from the epithelial surface of the vagina is a mixture of secretions from the Bartholin's glands and oviductal, follicular, and uterine fluids (13); hence the term "genital tract secretions" is, in fact, appropriate. Genital tract secretions contain a complex mixture of low- and high-molecular-weight components. Proteins and immunoglobulins are found in significant amounts ranging from 15 to 26 μg/ml (10). In addition, a variety of enzymes, such as proteases, are detected in vaginal fluids (3). Monosaccharides and polysaccharides, such as glucose and glycogen, have been detected among the carbohydrate components (15). The hemoglobin available during menses may be a source of iron for the growth of microorganisms that are otherwise iron limited (3). Aliphatic acids have also been reported to be present in these secretions as a by-product of microbial metabolism (2, 9). Analysis of the various ion components of vaginal fluid shows the presence of Na^+, K^+, and Cl^- in concentrations ranging from 20 to 60 mmol/liter (16).

In vitro test conditions comparable to the conditions in the human vagina are necessary for investigations of the specific mechanisms and factors that control microbial populations within the female genital tract. A first step in such in vitro studies is to develop a medium simulating in vivo conditions. This paper describes a defined medium with a composition similar to that of genital tract secretions for growth of vaginal microorganisms.

* Corresponding author.

CDM. The composition of the chemically defined medium (CDM) simulating female genital tract secretions for the growth of vaginal microflora is described in Table 1. Stock solutions in part I of the CDM were prepared by dissolving the components in the indicated amounts in 0.93 liters of deionized water. After adjustment of pH to 7.2 with NaOH, the solution was autoclaved for 15 min at 121°C and then cooled to room temperature. The components of parts II and III were then added to the vessel. Part II consisted of nine components, each of which was prepared separately. Hemin and vitamin K_1 solutions were prepared according to established procedures (4). Solutions 1 through 6 were autoclaved for 15 min at 121°C. Solutions 7 through 9 were sterilized by passage through a membrane filter (pore size, 0.22 μm). Part III consisted of a vitamin mixture as shown. Chemically defined agar medium (CDAM) was prepared for use in qualitative growth studies by the addition of 15 g of agar to 1 liter of CDM.

Microorganisms. The microorganisms tested in this study were isolates obtained from healthy volunteers by a vaginal swab technique (12). These organisms included *Bacteroides fragilis*, *Gardnerella vaginalis*, *Lactobacillus acidophilus*, *Peptostreptococcus asaccharolyticus*, *Prevotella intermedia*, *Prevotella bivia*, *Propionibacterium jensenii*, *Streptococcus intermedius*, and *Veillonella parvula*. Strains were maintained on prereduced brucella-based agar enriched with 5% sheep blood, hemin, and vitamin K_1 (BMB) (Adams Scientific Inc., Fiskeville, R.I.) at 36°C in an anaerobic chamber. *Candida albicans*, *Corynebacterium* sp., *Staphylococcus aureus*, and *Staphylococcus epidermidis* were maintained on tryptic soy agar with 5% sheep blood (TSB) (Adams Scientific) at 36°C under aerobic incubation conditions. Colonies were selected from BMB and TSB and streaked onto CDAM as a source of growth for inoculum preparations. The microorganisms were harvested with a swab, washed, and resuspended in phosphate-buffered saline (PBS) for quantitative growth studies.

Qualitative growth study. Colonies of bacteria were removed with a loop from CDAM cultures and inoculated onto fresh CDAM plates. Plates were incubated at 36°C in air for facultative species or in an anaerobic chamber for obligate anaerobes. Aerobic and anaerobic bacterial growth was recorded after 72 h and 7 days, respectively.

Quantitative growth study. CDM was used to study the growth rate and total populations for several vaginal isolates. Harvested cultures suspended in PBS were used to inoculate 5 ml of CDM fluid in screw-cap tubes. These tubes were incubated at 36°C in air for facultative species or in an

TABLE 1. Composition of CDM simulating genital tract secretions

Component	Amt (g/liter)	Stock solution (vol in ml)	Final concn
Part I[a]			
NaCl	3.5		0.06 M
KCl	1.5		0.02 M
K_2HPO_4	1.74		0.01 M
KH_2PO_4	1.36		0.01 M
Dextrose	10.8		0.06 M
Cysteine HCl	0.5		0.003 M
Part II[b]			
1. Glycogen		5.0% (20.00)	0.1%
2. Mucin		1.25% (20.00)	0.025%
3. Tween 20		2.0% (10.00)	0.02%
4. Urea		40.0% (1.25)	0.05%
5. Vitamin K_1		0.5% (0.20)	0.01%
6. Hemin		0.5% (10.00)	0.05%
7. Albumin		5.0% (40.00)	0.2%
8. $MgSO_4$		6.0% (5.00)	0.03%
9. $NaHCO_3$		4.0% (1.00)	0.004%
Part III[c]			
Biotin			0.005 mg/ml
myo-Inositol			50.0 mg/ml
Niacinamide			0.5 mg/ml
Pyridoxine HCl			0.5 mg/ml
Thiamine HCl			0.5 mg/ml
D-Calcium pantothenate			0.5 mg/ml
Folic acid			0.5 mg/ml
p-Aminobenzoic acid			0.01 mg/ml
Choline chloride			0.5 mg/ml
Ribofalvin			0.1 mg/ml
L-Ascorbic acid			1.0 mg/ml
Vitamin A (retinol)			0.005 mg/ml
Vitamin D (cholecalciferol)			0.005 mg/ml
Vitamin B_{12}			0.01 mg/ml

[a] All components from Fisher Scientific, Fair Lawn, N.J.
[b] Components 1, 2, and 4 through 7 from Sigma Chemical Co., St. Louis, Mo.; 3 from EM Science, Cherry Hill, N.J.; 8 and 9 from Fisher Scientific.
[c] A vitamin mixture (stock solution, 100X; volume, 5.0 ml) from Sigma Chemical Co. (K3129).

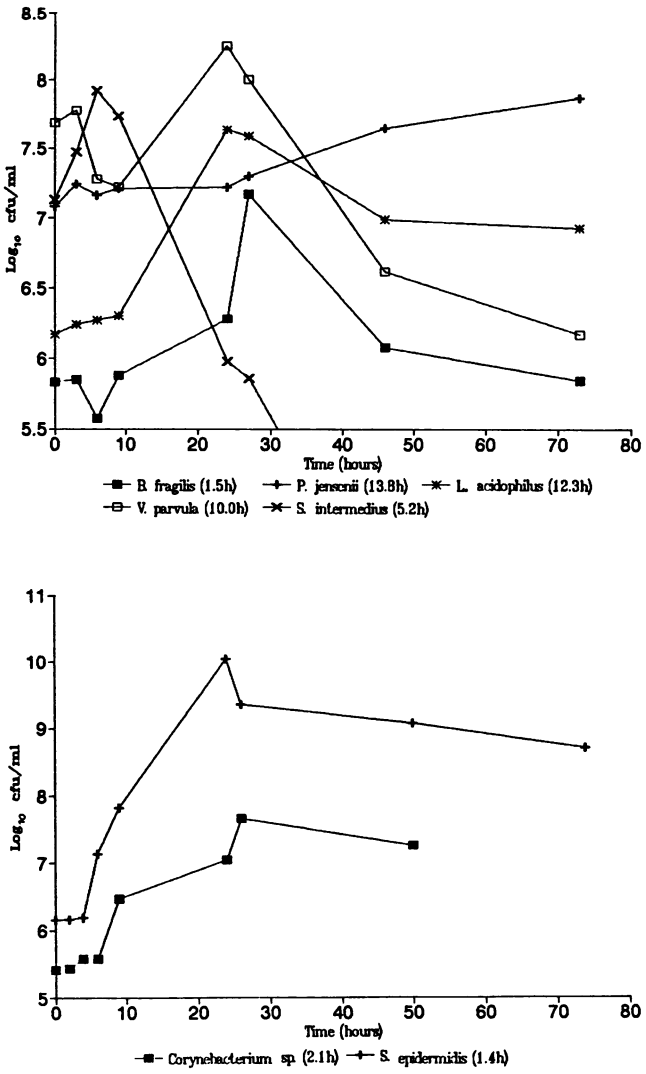

FIG. 1. Growth curve and maximum generation time in hours for vaginal isolates grown separately in CDM.

anaerobic chamber for obligate anaerobes. At various intervals, aliquots were removed for bacteriological analysis. Aliquots (0.1 ml) of undiluted cultures and of serial dilutions in PBS were plated onto TSB and BMB. Plates were incubated and growth was recorded as described above. Concentrations of organisms were expressed as $\log_{10}$ CFU per milliliter.

For in vitro studies of the complex interactions of the various microbial species that make up the vaginal microflora, a nutrient environment similar to that found in vivo is necessary. Such a medium must support the growth of all of the species that make up the vaginal microflora. We devised such a medium on the basis of published analyses of vaginal secretions. Initially, *L. acidophilus* and *S. epidermidis* were used to test the various growth-promoting characteristics of female genital tract secretions as potential constituents of this medium. At the conclusion of these studies, a CDM was formulated (Table 1), including the various components required for the growth of the principal constituents of the vaginal microflora. These components were used at concentrations approximating those detected in vaginal fluids by other investigators (10, 16). For some components the final concentration was modified in a manner that permitted the growth of microorganisms to levels consistent with those observed in vivo (11).

Our initial studies indicated that the presence of glycogen in the developed CDM prolonged the survival of *L. acidophilus* during batch growth. Glycogen and glucose are reported to be present in the vaginal environment at concentrations of 1.5 and 0.62 g%, respectively (15). These two carbohydrates are thought to be utilized in the vaginal environment mainly by *Lactobacillus* species (17). The presence of *Lactobacillus* species in relatively high numbers corresponds to the low pH of the healthy human vagina at times other than during menstrual flow (17). Potassium phosphate was added to the CDM to provide a buffering capacity for this medium.

The protein content of vaginal secretions was reported by Moghissi (10) to be 18 μg/ml; however, the methods used by Moghissi detect only a fraction of the amount of protein that is actually present; the portion that is not detected includes those proteins that are cellular components—primarily albumin, immunoglobulins, free amino acids, and serum-type proteins (10). Our initial studies indicated that a 2-mg/ml solution of albumin in the CDM was necessary to support the growth of most of the tested components of the vaginal microflora.

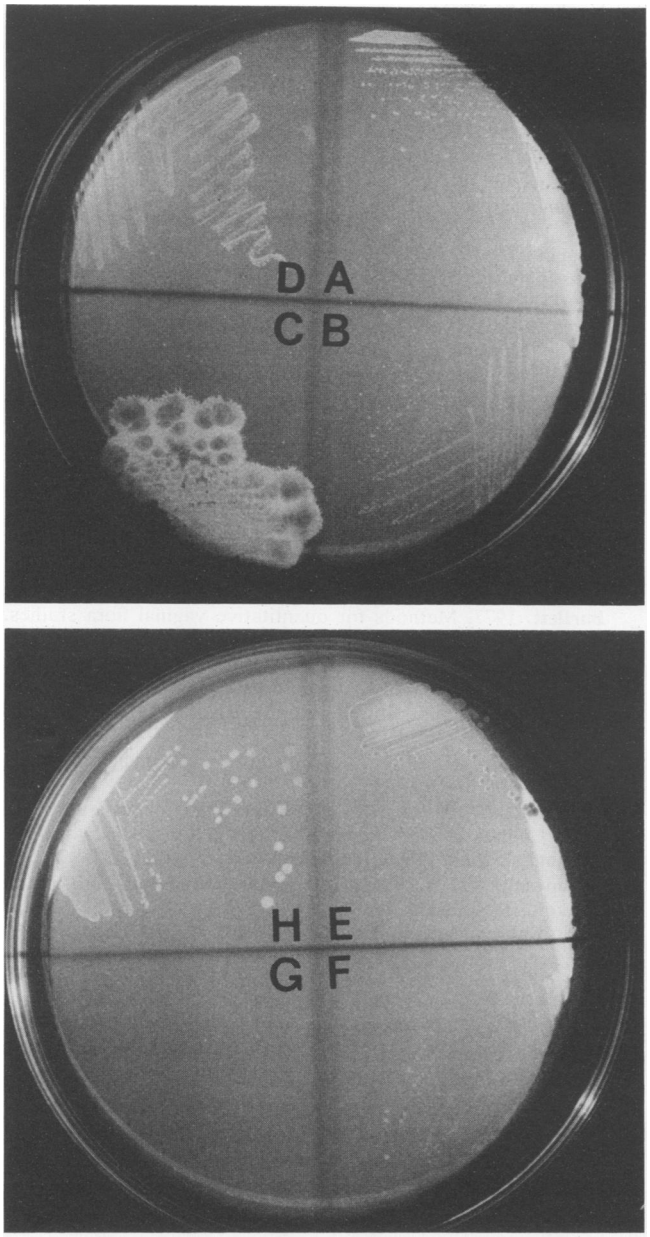

FIG. 2. Growth of *Staphylococcus aureus* (A), *Staphylococcus epidermidis* (B), *Candida albicans* (C), and *Corynebacterium* sp. (D) on CDAM incubated in air at 36°C for 72 h and of *Propionibacterium jensenii* (E), *Veillonella parvula* (F), and *Lactobacillus* sp. (H) (G is blank) incubated in an anaerobic chamber at 36°C for 6 days.

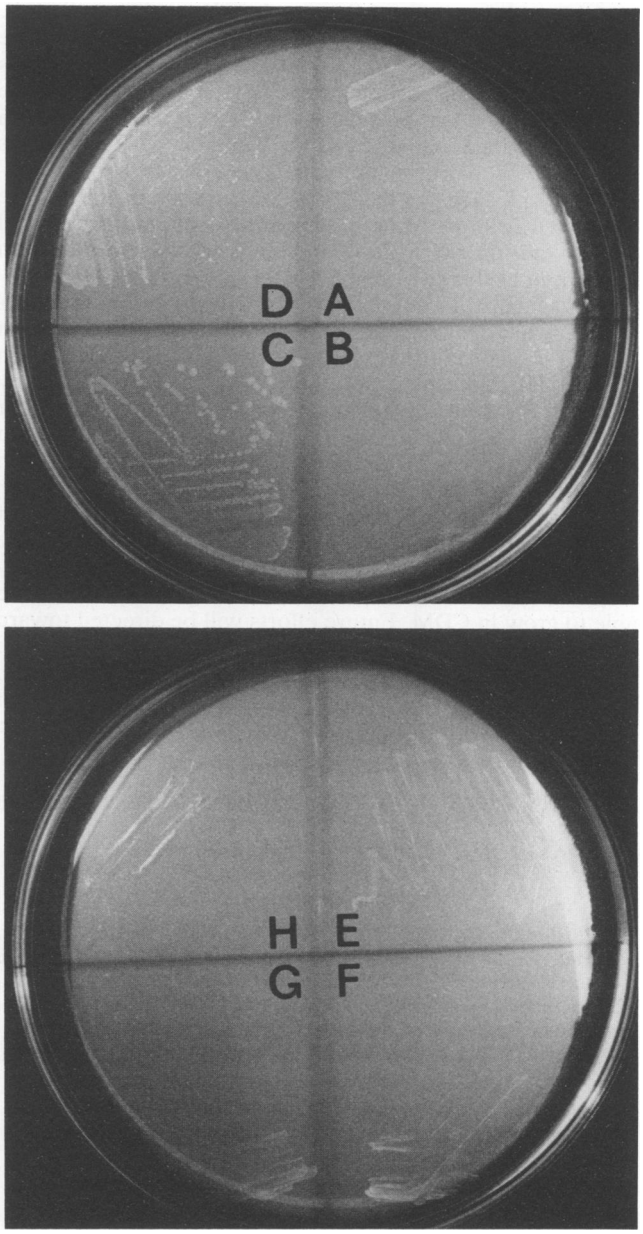

FIG. 3. Growth of *Lactobacillus acidophilus* (A), *Prevotella intermedia* (B), *Bacteroides fragilis* (C), and *Gardnerella vaginalis* (D) on CDAM and of *B. fragilis* (E), *Peptostreptococcus* sp. (F), and *Lactobacillus* sp. (G) (H is blank) incubated in an anaerobic chamber at 36°C for 6 days.

The reported concentrations of Na^+, K^+, and Cl^- in vaginal fluid are approximately 23, 61, and 62 mmol/liter, respectively (16). These concentrations were adequate to support growth of the microorganisms tested. Urea has been detected at a concentration of 49 mg/100 ml in vaginal fluid (10). Mucin has also been reported to be present as a high-molecular-weight component of vaginal secretions (13). A mucin concentration of 0.025% was employed in the CDM.

Cysteine was used as a reducing agent to establish the E_h of the vaginal environment for the growth of the vaginal microflora (5). Polyoxyethylene sorbitan monooleate (Tween 20) was added to the CDM as a source of long-chain fatty acids as well as a growth stimulant for *Lactobacillus* species (8).

Although published reports do not indicate the presence of other growth factors in vaginal fluids, most microorganisms require some additional growth factors in an in vitro environment. The supplementation of the CDM with growth factors (see Table 1) provides more suitable conditions for the growth of vaginal microorganisms in vitro (8).

Various vaginal isolates were examined during a quantitative evaluation of growth to determine both maximal achievable populations and maximal growth rate. In addition, *P.*

bivia, *P. intermedia*, and *P. asaccharolyticus*, as well as *G. vaginalis* and *C. albicans*, were evaluated along with these isolates in qualitative tests to determine whether fastidious organisms and yeasts could grow in this medium. During the quantitative evaluation, CDM fluid was inoculated with vaginal isolates, and the viable bacterial cell count was determined at various intervals. Growth curves were obtained by plotting the data (Fig. 1). Generation times were calculated from the time required for doubling of bacterial density during the logarithmic growth phase. Maximum generation at this pH ranged from 1.4 to 13.8 h.

All vaginal isolates grew well on CDAM during the qualitative evaluation. Figures 2 and 3 show examples of growth of the various vaginal isolates under appropriate incubation conditions. After incubation for 72 h in air, the colony diameter was generally 0.5 mm (2 mm for *C. albicans*). After incubation for 7 days, isolates requiring anaerobic growth conditions grew well and formed colonies that ranged in diameter from 0.5 to 1 mm.

In this study, attention was focused on the ability of vaginal microorganisms to grow in a CDM simulating female genital tract secretions. The results indicate that representative strains of the predominant vaginal species were able to grow in CDM. Future efforts will be directed toward the use of CDM for in vitro studies of issues such as the cyclic pH changes in this polymicrobial system. In addition, CDM may be useful as a medium for continuous cultures of the vaginal microflora with growth rates approximating those noted in vivo.

We acknowledge the valuable technical assistance of Merideth Talbot.

This research was funded by a grant from Tambrands, Inc.

REFERENCES

1. **Bartlett, J. G., A. B. Onderdonk, E. Drude, C. Goldstein, M. Anderka, S. Alpert, and W. M. McCormack.** 1977. Quantitative bacteriology of the vaginal flora. J. Infect. Dis. **136**:271–277.
2. **Bonsall, R. W., and R. P. Michael.** 1971. Volatile constituents of primate vaginal secretions. J. Reprod. Fertil. **27**:478–479.
3. **Cohen, M. S., J. R. Black, R. A. Proctor, and P. F. Sparling.** 1984. Host defences and the vaginal mucosa. Scand. J. Urol. Nephrol. **86**:13–22.
4. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed., p. 144–148. Virginia Polytechnic Institute and State University, Blacksburg.
5. **Holmes, K. K., K. C. S. Chen, C. M. Lipinski, and D. A. Eschenbach.** 1985. Vaginal redox potential in bacterial vaginosis (nonspecific vaginitis). J. Infect. Dis. **152**:379–382.
6. **Huggins, G. R., and G. Preti.** 1976. Volatile constituents of human vaginal secretions. Am. J. Obstet. Gynecol. **126**:129–136.
7. **Huggins, G. R., and G. Preti.** 1981. Vaginal odors and secretions. Clin. Obstet. Gynecol. **24**:355–377.
8. **Kandler, O., and N. Weiss.** 1986. Genus *Lactobacillus* Beijerinck 1901, 212^{AL}, p. 1209–1219. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
9. **Michael, R. P., R. W. Bonsall, and M. Kutner.** 1975. Volatile fatty acids, "coplins," in human vaginal secretions. Psychoneuroendocrinology **1**:153–163.
10. **Moghissi, K. S.** 1979. Vaginal fluid constituents, p. 13–23. *In* F. K. Beller and G. F. B. Schumacher (ed.), The biology of the fluids of the female genital tract. Elsevier/North-Holland Biomedical Press, Amsterdam.
11. **Onderdonk, A. B., B. F. Polk, N. E. Moon, B. Goren, and J. G. Bartlett.** 1977. Methods for quantitative vaginal flora studies. Am. J. Obstet. Gynecol. **122**:777–781.
12. **Onderdonk, A. B., G. R. Zamarchi, M. L. Rodriguez, M. L. Hirsch, A. Muñoz, and E. H. Kass.** 1987. Quantitative assessment of vaginal microflora during the use of tampons of various compositions. Appl. Environ. Microbiol. **53**:2774–2778.
13. **Paavonen, J.** 1983. Physiology and ecology of the vagina. Scand. J. Infect. Dis. Suppl. **40**:31–35.
14. **Spiegel, C. A., R. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes.** 1980. Anaerobic bacteria in nonspecific vaginitis. N. Engl. J. Med. **303**:601–607.
15. **Sumawong, V., A. T. Gregori, W. D. Johnson, and A. E. Roakoff.** 1962. Identification of carbohydrates in the vaginal fluid of normal female. Fertil. Steril. **13**:270–280.
16. **Wagner, G., and R. J. Levin.** 1980. Electrolytes in vaginal fluid during the menstrual cycle of coitally active and inactive women. J. Reprod. Fertil. **60**:17–27.
17. **Wylie, J., and A. Henderson.** 1969. Identity and glycogen-fermenting ability of lactobacillus isolated from the vagina of pregnant women. J. Med. Microbiol. **2**:363–366.