

Planktonic or Biofilm *Streptococcus agalactiae* Antagonize Probiotic and Indigenous Vaginal Lactobacilli in vitro

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Abstract

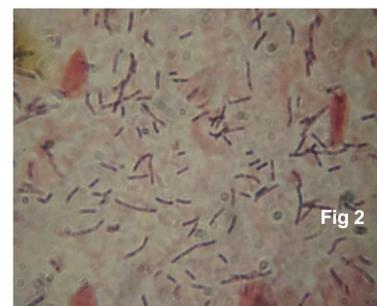
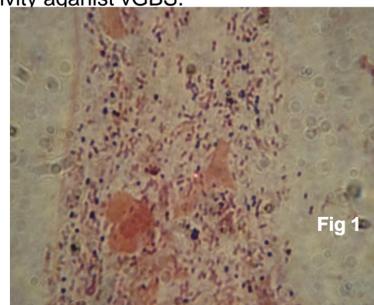
Abstract: Background: *Streptococcus agalactiae* is part of vaginal flora (vGBS) but is a common cause of infectious diseases in newborn and in pregnant/non pregnant adults. Vaginal lactobacilli (vLB) exert health benefits through probiotic actions. Vagina is an ecosystem where microbes could be found in planktonic (PI) and biofilm (Bf) status. We study the interaction between PI and Bf vGBS and vLB *in vitro*. **Methods:** We use 4 vGBS isolates (2 pathogenic and 2 colonizing) from sexually active women (SAW), 3 vLB from healthy SAW and probiotic *Lactobacillus fermentum* ATCC 9338. (LF). PI growth and Bf formation were performed in these media: Man Rogosa Sharpe (MRS), Todd Hewitt (TH), ChromidID StreptoB agar (STRB) (bioMérieux), Tryptic Soy (TS) and blood TS (BTS). Overlay methods were used for PI interaction between vGBS and vLB or LF. Both were grown either in bottom or upper layer using MRS, STRB and BTS agar. For Bf, vGBS or vLB/LF were grown on glass coupons as we previously describe using MRS, TH and TS broth. We challenge each Bf vGBS with all vLB/LF and viceversa. Cocultures for Bf formation were assayed.

Results: In PI conditions 3/4 vGBS fully inhibited and 1/4 partially inhibited LF growth. The colonizing vGBS inhibited partially or totally vLB growth while pathogenic vGBS only inhibited vLB growth partially. In BTS overlay growth an enhanced β -hemolysis by all vGBS was observed. vLB/LF didn't inhibit the vGBS growth under PI conditions. In Bf experiments when sessile vGBS was first established it seems to antagonize the vLB/LF adherence, impairing formation of mixed Bf. When vLB/LF Bf was first developed, vGBS displace sessile lactobacilli. In cocultures, vGBS predominate over vLB/LF in the sessile form.

Conclusions: Our study indicated that vLB/LF could not fully prevent colonization by vGBS *in vitro* and it is isolate dependent. This antagonizing behaviour between vLB/LF and vGBS, either in PI or Bf conditions, seems to show that the use of probiotics to prevent or diminished vGBS colonization in women could be useless.

Background

The vaginal ecosystem is a complex environment in which lactobacilli are the most predominant bacterial species, therefore, they have been used extensively as a vaginal probiotic, to prevent infections from other bacterial species. Also, *Streptococcus agalactiae* is present as vaginal normal flora in 15-25% of normal and asymptomatic women. However vGBS can produce aerobic vaginitis in sexually active and postmenopausal women. (Fig 1). At the same time GBS in pregnant women can be transmitted vertically and produce early-onset GBS sepsis mainly when risk factors are present: pre-delivery fever, PROM, pPROM, and preterm delivery. Probiotic lactobacilli have had some success because their receptors on vaginal cells are glycolipids, which presumably were the targets for the competition observed between lactobacilli and pathogenic microbes. But so far, there are no reports on its activity against vGBS. Probiotic Lactobacilli have had some success because their receptors on vaginal cells are glycolipids, which presumably were the targets for the competition observed between lactobacilli and pathogenic microbes. But so far, there are no reports on its activity against vGBS.



Objective

To study the interaction between PI and Bf vGBS and vLB (Fig 2) *in vitro* for evaluating probiotic activity in vagina

Methods

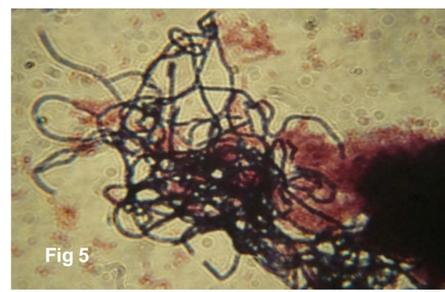
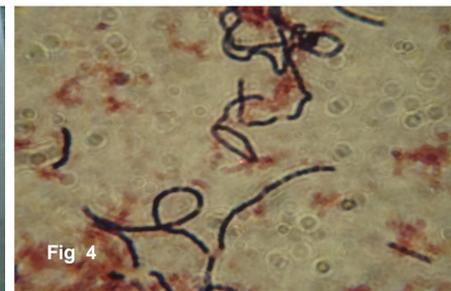
Strains: We use 4 vGBS isolates (2 pathogenic and 2 colonizing) from sexually active women (SAW), 3 vLB from healthy SAW and probiotic *Lactobacillus fermentum* ATCC 9338. (LF).

Media: The different strains were cultured for PI growth or Bf formation in Man Rogosa Sharpe (MRS), Todd Hewitt (TH), ChromidID StreptoB agar (STRB) (bioMérieux), Tryptic Soy (TS) and blood TS (BTS).

Overlay technique: the microorganisms were grown either in bottom or upper layer using MRS, STRB and BTS agar.

Planktonic cocultures: first vGBS or vLB/LF Bf were first grown either in MRS or TS broth up to stationary conditions. Then equal amounts of microorganisms were coinoculated in respective broth, grown for 72 hs and then plated in MRS and BTS to study lactobacilli presence.

Biofilm experiments: vGBS or vLB/LF Bf were developed on glass coupons (Fig 3, 4 and 5) as we previously describe using MRS, TH and TS broth. We challenge each Bf vGBS with all vLB/LF and viceversa.



Results

Overlay technique

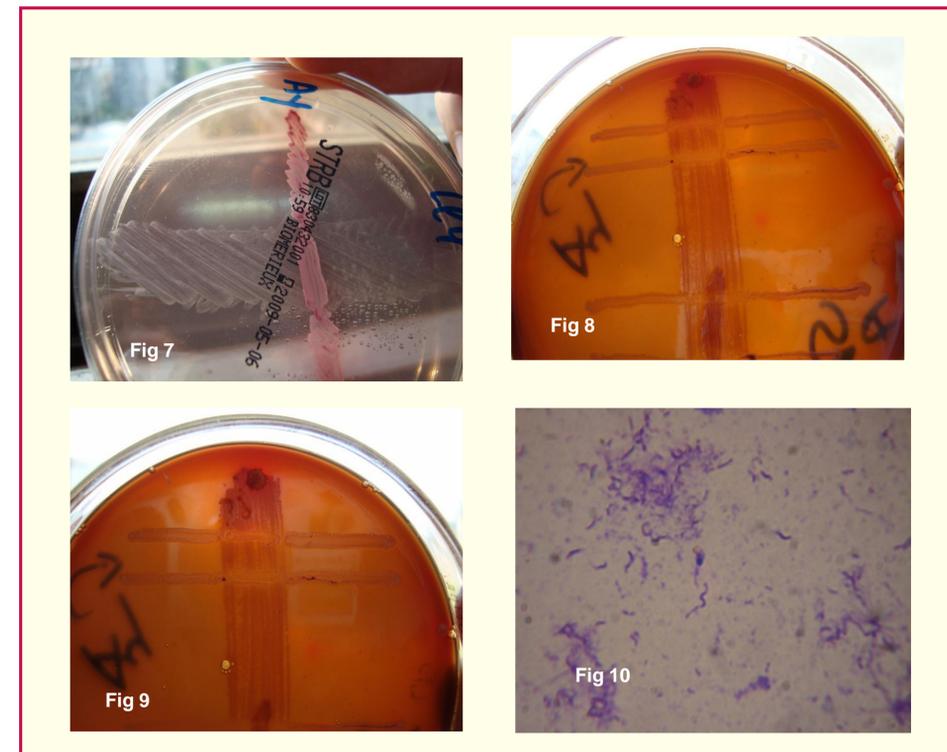
This method was used to study PI interaction between vGBS and vLB or LF. The results indicated that vLB/LF didn't inhibit vGBS growth (Fig 7). Also an enhanced β -hemolysis by all vGBS was observed when BTS was used as bottom layer (Fig 8 and 9), probably indicating an enhance in their virulence.

Planktonic cocultures

We performed this experience to check how GBS interact with lactobacilli under planktonic conditions. Our results indicated that GBS fully or partially inhibited LB growth. (Table 1) Also, we verify under these conditions that neither vaginal LB from healthy sexually active women nor probiotic *Lactobacillus fermentum* (vLB/LF) inhibit the vGBS growth (Fig 6)

Biofilm experiments

In Bf experiments when sessile vGBS was first established it seems to antagonize the vLB/LF adherence, impairing formation of mixed Bf. When vLB/LF Bf was first developed, vGBS displace sessile lactobacilli. In cocultures, vGBS predominate over vLB/LF in the sessile form. (Fig 10)



vGBS	Ay-pathogenic	Al-pathogenic	A-colonizing	B-colonizing
LF ATCC 9338	MRS - TS -	MRS - TS -	MRS - TS -	MRS +/- TS -
vLB	MRS +/- TS +/-	MRS +/- TS +/-	MRS +/- TS +/-	MRS +/- TS +/-

Conclusions

GBS in pregnant women can be transmitted vertically and produce early-onset GBS sepsis mainly when risk factors are present: pre-delivery fever, PROM, pPROM, and preterm delivery.

Probiotic lactobacilli are commonly used during pregnancy to prevent bacterial vaginosis and other endogenous infections. Nevertheless, no report has been published until now about the ability of these probiotics to block vGBS activity.

Our study indicated that vLB or LF could not fully prevent colonization by vGBS *in vitro*. Also, in some cases, the presence of Lactobacilli, seems to improve the virulence of GBS. This antagonizing behaviour between vLB/LF and vGBS, either in PI or Bf conditions, could indicate that the use of probiotics to prevent or diminished vGBS colonization in women could be harmful.