Social interaction by BV anaerobes in initial adhesion and biofilm assays

A. MACHADO[1,2], D. SALGUEIRO[1], L. RODRIGUES[1], N. CERCA[1] AND K. JEFFERSON[2]

[1] IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057, Braga, Portugal
[2] Department of Microbiology and Immunology, PO Box 980678, Virginia Commonwealth University, Richmond, VA 23928, USA

Introduction

Bacterial vaginosis (*BV*) is a common vaginal disorder of women of reproductive age. It is commonly accepted that the microbial switch from normal to BV state is characterized by a decrease in vaginal colonization by *Lactobacillus* species together with an increase in the number of *Gardnerella vaginalis* (*GV*) and other anaerobes.

GV can form a biofilm on vaginal epithelium and this appears to be involved in the pathogenesis of BV and in antibiotic failure. *GV* biofilms may also facilitate growth of other anaerobes. We hypothesized that an important role for vaginal lactobacilli in the maintenance of vaginal health is prevention of biofilm formation. Further research into the *GV* interactions with other anaerobes may be crucial to understand *BV* etiology.

Therefore, our goal was to study competitive initial adhesion between *L. crispatus* and several *BV* anaerobes. Also, this study aimed to evaluate differences in biofilm formation between a *GV* strain from a healthy subject (5-1) and the other anaerobes as compared with a *BV GV* strain (101). In addition to *GV* strains, *P. bivia*, *F. nucleatum*, *M. mulieris* and *A. vaginae* were used in this investigation of bacterial social interaction.

Methods

Initial adhesion competitive assays through FISH analysis

1. Initial adhesion at 100 rpm for 30 min was conducted in glass 8-well slides, using an equal mixture of *L. crispatus* 39G strain and anaerobe (*GV* 5-1 and 101, *P. bivia*, *F. nucleatum*, *M. mulieris* and *A. vaginae*) at same concentration (10^3 CFU/ml);
2. Each well was washed with PBS and fluorescently stained with a lactobacilli specific probe and DAPI;
3. Bacteria counting of the adhesion was done by 20-fields enumeration in each well.

Biofilm formation assays through quantitative PCR analysis

1. Mixed biofilm formation between a pre-established (24h) GV strain biofilm and a second inoculation by other anaerobe until reached 48h incubation (37ºC at 5%CO_2);
2. Each well was washed with PBS and DNA extraction was done by an enzymatic kit;
3. Quantitative PCR was realized with specific primers for each bacteria for 40 cycles.

Results

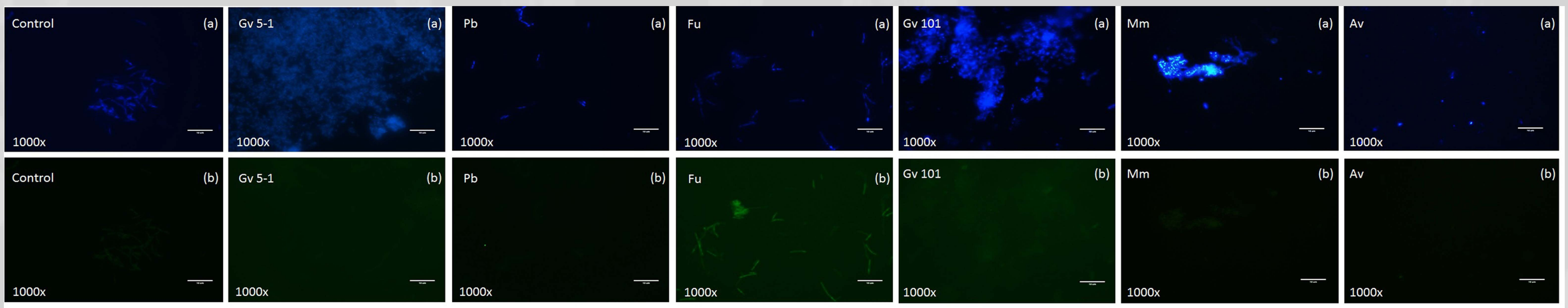

Figure 1 - Fluorescence microscopy of the initial adhesion competitive assays between *Lactobacillus crispatus* and a second anaerobe at equal low level of concentration (10^3 CFU/ml) in 8-well glass slides by 4',6-diamidino-2-phenylindole (DAPI) and specific PNA probe (Lac663) associated with Alexa Fluor 488 fluorochrome.

Legend – (a) blue filter; (b) green filter; Control, *L. crispatus*; Gv 5-1, *G. vaginalis* 5-1 *& L. crispatus*; Pb, *P. bivia & L. crispatus*; Fu, *F. nucleatum & L. crispatus*; Gv 101, *G. vaginalis* 101 *& L. crispatus*; Mm, *M. mulieris & L. crispatus*; Av, *A. vaginae & L. crispatus*.

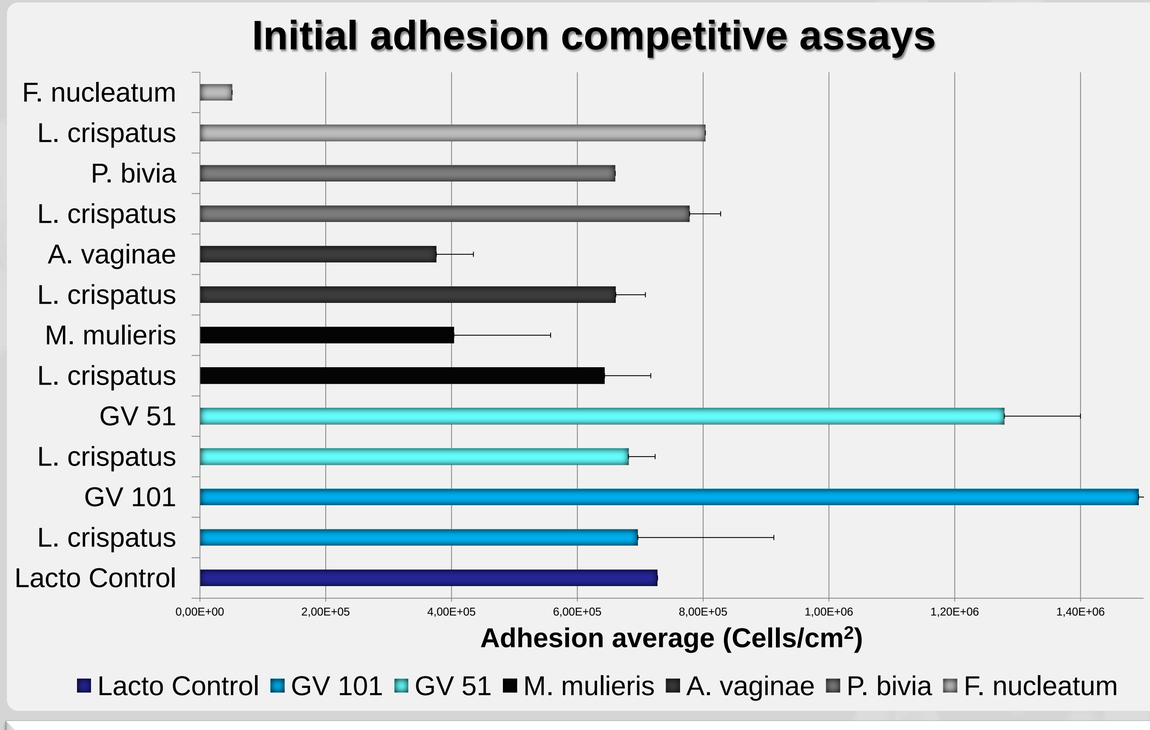

Figure 2 – Initial adhesion competitive assays realized by equal mixture between *Lactobacillus crispatus* 39G and a second anaerobe at low level each one (10^3 CFU/ml) in PBS buffer solution during 30min at 100rpm and anaerobic conditions.

Primers	Biofilm formation samples	qPCR CT	Primers	Biofilm formation samples	qPCR CT
Fusobacterium	Fu for 24h	25.12	Fusobacterium	Fu for 24h	24.24
Fusobacterium	Gv 5-1 for 48h & Fu for 24h	24.42	Fusobacterium	Gv 101 for 48h & Fu for 24h	23.47
Prevotella	Pb for 24h	23.50	Prevotella	Pb for 24h	24.84
Prevotella	Gv 5-1 for 48h & Pb for 24h	21.93	Prevotella	Gv 101 for 48h & Pb for 24h	22.90
Atopobium	Av for 24h	25.78	Atopobium	Av for 24h	26.38
Atopobium	Gv 5-1 for 48h & Av for 24h	25.60	Atopobium	Gv 101 for 48h & Av for 24h	26.07
Mobiluncus	Mm for 24h	31.80	Mobiluncus	Mm for 24h	31.99
Mobiluncus	Gv 5-1 for 48h & Mm for 24h	30.56	Mobiluncus	Gv 101 for 48h & Mm for 24h	32.15
Gardnerella	Control 48h (no bacteria)	30.41	Gardnerella	Control 48h (no bacteria)	31.09
Gardnerella	Gv 5-1 for 48h alone	14.51	Gardnerella	Gv 101 for 48h Alone	14.13
Gardnerella	Gv 5-1 for 48h & Mm for 24h	13.17	Gardnerella	Gv 101 for 48h & Mm for 24h	12.23
Gardnerella	Gv 5-1 for 48h & Av for 24h	13.20	Gardnerella	Gv 101 for 48h & Av for 24h	12.41
Gardnerella	Gv 5-1 for 48h & Pb for 24h	13.39	Gardnerella	Gv 101 for 48h & Pb for 24h	12.20
Gardnerella	Gv 5-1 for 48h & Fu for 24h	14.20	Gardnerella	Gv 101 for 48h & Fu for 24h	12.29

Table 1 – Results of the quantitative PCR (qPCR) from mixed biofilm formation assays with *Gardnerella vaginalis* strains (5-1 and 101) and a second BV anaerobe.
Legend – CT, threshold cycle; TGv 5-1, *G. vaginalis* 5-1; Gv 101, *G. vaginalis* 101; Mm, *M. mulieris*; Av, *A. vaginae*; Pb, *P. bivia*; Fu, *F. nucleatum*.
All the experiments were realized in triplicate.

Conclusions

Our results suggest that *G. vaginalis* 5-1 and 101 strains had the greatest initial adherence capability in presence of an equal number of *L. crispatus* cells, followed by *P. bivia*, *M. mulieris* and *A. vaginae*.

In addition, qPCR analysis of the two species biofilms revealed that both *G. vaginalis* strains established commensal relationships with all others anaerobes except with *P. Bivia*, for which a synergistic relation was found. In fact, a synergistic relationship between these two species had previously been noted. Importantly, *G. vaginalis* 101 (pathogenic strain) showed nearly a 2-fold increase in biofilm formation when compared with *G. vaginalis* 5-1 (healthy strain) in the presence of any other anaerobe studied.

In conclusion, this study revealed potentially relevant differences between the adherence and biofilm formation of *BV* and healthy *G. vaginalis* isolates in the presence of *L. cripatus* and other *BV* well-know anaerobes. Further research in these *GV* strains and their social interactions with other BV anaerobes may be crucial for better understanding of the *BV* etiology.

Acknowledgments: This work was supported by European Union funds (FEDER/COMPETE) and by national funds (FCT) under the project with reference FCOMP-01-0124-FEDER-008991 (PTDC/BIA-MIC/098228/2008). AM acknowledges the FCT individual fellowship – SFRH/BD/62375/2009.