

119 Social interaction by BV anaerobes in initial adhesion and biofilm assays

Antonio Machado^{a,b}; Débora Salgueiro^a; Ligia Rodrigues^a; Nuno Cerca^{a,*} Kimberly Jefferson^b

^aIBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057, Braga, Portugal. ^bDepartment of Microbiology and Immunology, PO Box 980678, Virginia Commonwealth University, Richmond, VA 23928, USA. *Corresponding author: nunocerca@ceb.uminho.pt

Bacterial vaginosis (BV) is the most 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* and others anaerobes. *G. vaginalis* can be found associated with normal vaginal epithelium but recent findings suggest that it is the biofilm produced by *G. vaginalis* that leads to establishment of BV, by allowing other anaerobes to grow. Further research into the properties of *G. vaginalis* and its interactions with other BV-associated anaerobes may be essential for better understanding the BV etiology. Therefore, our goal was to study competitive initial adhesion between *Lactobacillus crispatus* (an important vaginal species with probiotic activity) and several anaerobes. Also, this study aimed to evaluate differences in biofilm formation between a healthy *G. vaginalis* strain (5-1) and the other anaerobes as compared with a BV *G. vaginalis* strain (101). In addition to *G. vaginalis* strains (5-1 and 101), *Prevotella bivia*, *Fusobacteria nucleatum*, *Mobiluncus mulieris* and *Atopobium vaginae* were used in this social interaction research. First, initial adhesion assays at 100rpm for 30 min were conducted in glass 8-well slides using an equal mixture of *L. crispatus* 39G strain and a second anaerobe at same concentration (10^3

CFU/ml) in each well. Next, we used qPCR to study the biofilm social evolution (synergic, antagonist and commensal relationship) between a pre-established (24h) biofilm formed by one of the two *G. vaginalis* strains and a second anaerobe. Our results showed that *G. vaginalis* 5-1 and 101 strains had definitively the greatest initial adherence capability when in presence of the same number of *L. crispatus* 39G 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 synergic relation was found. In fact, this synergic relation 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.

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).

Keywords: Bacterial vaginosis, mixed species biofilms, FISH, qPCR.