P224. *Gardnerella vaginalis* enhances *Atopobium vaginae* viability *in vitro*

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Background
Bacterial vaginosis (BV) is a clinical condition characterized by a dramatic shift in the vaginal microflora from the beneficial lactobacilli to a polymicrobial flora, consisting of strictly and facultatively anaerobic bacteria. It is noteworthy that a hallmark of BV is the presence of a highly structured polymicrobial biofilm on the vaginal epithelium, presumably initiated by a facultative anaerobe, *Gardnerella vaginalis*, which then become a scaffold for other species to adhere. While not much is known about multi- species interactions within BV biofilms, *Atopobium vaginae* is often associated with *G. vaginalis* biofilms and is rarely detected without *G. vaginalis*.

Methods
This study assessed interactions between *G. vaginalis* and *A. vaginae*, analyzing both mono- and dual-species cultures. Firstly, we evaluated the impact of *A. vaginae* on a pre-established *G. vaginalis* biofilm, by determining the total biofilm biomass using the crystal violet method. Furthermore, the bacterial distribution and biofilm structure were evaluated by Peptide Nucleic Acid Fluorescence in situ Hybridization (PNA-FISH) and confocal laser scanning microscopy analysis. Afterward, quantification of viable bacteria within pure or dual-species planktonic cultures was performed by using specific *G. vaginalis* or *A. vaginae* PNA probes, using the FISH method.

Results and Conclusion
We observed that, in our in vitro conditions, *A. vaginae* was not able to establish a single-species biofilm but easily incorporated a pre-formed *G. vaginalis* biofilm. Interestingly, *A. vaginae* lost viability after 48 hours of single-species planktonic growth but was able to maintain viability when co-cultured with *G. vaginalis*. This demonstrated that in vitro *A. vaginae* is dependent on *G. vaginalis* to survive, providing an explanation of the co-occurrence of these two species *in vivo*. Overall, this study underlined the importance of the ecological interactions between these BV-associated species, which might delineate the development of BV.

Acknowledgments
This study was supported by the Portuguese Foundation for Science and Technology (FCT) by the funded project PTDC/BIA-MIC/28271/2017, under the scope of COMPETE2020 (POCI-01-0145- FEDER-028271) and by the strategic funding of unit UID/BIO/04469/2019.