

Lactobacilli and its metabolites as potential probiotics against *Gardnerella vaginalis*

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Bacterial vaginosis (BV) is one of the most common gynecological disorder affecting women in reproductive age. This condition poses a significant health risk because it predisposes women to abnormal pregnancy, pelvic inflammatory disease and an increased risk of sexual transmitted infections, including HIV [1]. Although BV etiology remains unknown, it is characterized by a decrease in vaginal lactobacilli and a proliferation of anaerobes [1, 2]. Despite of the richness and diversity found into BV anaerobes, *Gardnerella vaginalis* is present in over 90% of the pathologic cases and several studies report its potential as the main etiological candidate [3, 4, 5]. Current BV treatment is strictly based in antibiotic therapy resulting in an increased resistance of BV anaerobes, along with severe reduction of the healthy lactobacilli strains in the vaginal epithelium. Therefore, a more appropriate treatment is required, aiming to decrease *G. vaginalis* and also to promote the lactobacilli re-colonization in BV patients [6]. Previous studies showed the potential of lactobacilli in preventing vaginal colonization by pathogens, and thus the development of infections, by different mechanisms including auto-aggregation, co-aggregation with pathogenic microorganisms, and adhesion to epithelial cells and/or by producing some metabolites (such as lactic acid, hydrogen peroxide, bacteriocins and biosurfactants) that may act as growth inhibitors or anti-adhesive agents [7]. These probiotic properties have inspired new treatment strategies for vaginal infection.

One alternative therapy for BV is the re-colonisation of vagina with lactobacilli species [6]. However, the major gap in this option resides in the choice of the most suitable lactobacillus species. Our goal was to evaluate the probiotic potential of intra and extracellular metabolites from a broad range of lactobacilli strains against several *G. vaginalis* strains. To accomplish our goal, we isolated 60 vaginal lactobacilli strains from healthy women and performed a screening by an agar spot test against 9 *G. vaginalis* strains (3 from culture collection and 6 clinical isolates from women with BV) in order to selected the most interesting probiotic candidates. We also included more than 30 culture collection lactobacilli strains. For the best candidates we determined the minimum inhibitory concentration (MIC) and identified the lactobacilli metabolites responsible for *G. vaginalis* growth inhibition.

Our results showed that certain latobacilli metabolites were able to inhibit *G. vaginalis* growth. In spite of the detection of intracellular biosurfactants in some strains, they were unable to reduce *G. vaginalis* proliferation. On the other hand, extracellular products of some lactobacilli showed a significant effect on *G. vaginalis* growth. Overall, from the 90 lactobacilli strains tested, only 4 culture collection and 3 clinical isolate lactobacilli strains exhibited a broad probiotic activity against all the *G. vaginalis* strains tested. However, only culture collection strains were able to reduce *G. vaginalis* strains growth to 20-30% in MIC assays, illustrating an efficient probiotic activity by itself. Interestingly, none of these lactobacilli strains belong to the vaginal microflora, revealing a more pronounced probiotic activity than any of those vaginal isolate lactobacilli tested.

Therefore, our data suggests the existence of non-vaginal lactobacilli strains possessing probiotic activity against numerous *G. vaginalis* strains, which may contribute for a new and more effective BV treatment than the currently used therapies.

Keywords: Bacterial vaginosis, *Gardnerella vaginalis*, lactobacilli, antimicrobial activity, probiotic.

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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).