

Probiotic potential of novel Brazilian *Lactobacillus crispatus* strains

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ABSTRACT. Lactobacilli are the predominant bacterial species colonizing the vaginal surfaces of healthy women, where they play a protective role against opportunistic and polymicrobial infections, such as bacterial vaginosis. Several *Lactobacillus* species, especially *L. crispatus*, have been prospected for probiotic applications due to their potential antimicrobial and anti-inflammatory capacities. During the last decade, several genomic studies have been investigating the genetics of *L. crispatus* strains in an effort to identify novel probiotic strains and evaluate their potential for improving human and animal health. This mini review highlights the main genes associated with *L. crispatus* protective mechanisms in four novel strains of this species that we recently isolated from healthy Brazilian women of reproductive age. Among the probiotic features of these strains, the roles of a pyruvate oxidase-encoding gene, lactate synthesis related enzymes, bacteriocin genes, and genomic islands, are reviewed, and the next steps for confirming their activity are indicated.

INTRODUCTION

First identified in 1894 by a German physician named Doderlein, lactobacilli have been reported as the dominant bacterial species that colonize the vaginal epithelium of women of reproductive age (Tachedjian et al., 2018). *Lactobacillus crispatus* is the most frequently isolated microorganism from this environment; it plays an important role in

protecting the host from potentially pathogenic bacteria naturally found in the vagina, which can cause polymicrobial synergistic infections known as bacterial vaginosis. These pathogenic microorganisms may include *Enterococcus faecalis*, *Gardnerella vaginalis*, *Prevotella bivia*, and other species (Castro et al., 2019). Due to the protective role of this species, *L. crispatus* strains are considered good candidates for probiotic use. Genomic studies have been useful in identifying genetic factors associated with beneficial properties for screening for probiotic features. This short review focuses on the main protective mechanisms and associated genes in four previously identified *L. crispatus* strains (Almeida et al., 2021) that had been isolated from healthy Brazilian women.

Hydrogen peroxide antimicrobial activity

Vaginal lactobacilli are aerotolerant anaerobes and may produce hydrogen peroxide (H_2O_2) *in vitro* when cultivated under aerobic conditions (Tachedjian et al., 2018). H_2O_2 is mainly formed in carbon and energy metabolism by the activity of oxidases, including pyruvate oxidase, lactate oxidase, and NADH oxidases. Increased levels of this metabolite is generally associated with species lacking H_2O_2 -scavenging enzymes, such as catalase, which leads to its accumulation within the cell and in the environment around the cell (Hertzberger et al., 2014). Studies have suggested that H_2O_2 -producing *Lactobacillus* strains present antimicrobial activity against bacterial vaginosis related pathogens, such as *G. vaginalis*, *P. bivia*, and *Candida albicans*; the physiological concentrations of H_2O_2 (0.05 to 1.0 mM) they produce play an important role, along with other substances produced by *Lactobacillus* spp. (Matu et al., 2010). Association studies have shown a positive correlation between H_2O_2 -producing strains of *Lactobacillus* spp. and healthy vagina clinical outcomes, suggesting this metabolite as a marker for probiotic strains. Almeida et al. (2021) identified four strains of *L. crispatus* that have a pyruvate oxidase-encoding gene; they were isolated in Brazil from healthy women of reproductive age. Although these strains appear to have potential as probiotics, further research will be required to examine the amount of H_2O_2 produced and antimicrobial activity.

It has also been suggested that H_2O_2 has anti-inflammatory activity in host cells by activating the peroxisome proliferator activated receptor γ (PPAR- γ), which plays a key role in the regulation of intestinal inflammation and homeostasis. In this context, proof-of-concept studies carried out *in vivo* using animal models for gastrointestinal inflammatory diseases would be useful (Hertzberger et al., 2014).

Other studies have shown that H_2O_2 -mediated inhibition can be neutralized through production of catalase by pathogens (Zheng et al., 1994; St Amant et al., 2002). Consequently, investigating antimicrobial activity that results from increased acidity and production of proteases and bacteriocins by lactobacilli is also important.

Bacteriocins and other antimicrobial peptides

Helping promote vaginal ecosystem restoration, besides producing organic acids and hydrogen peroxide, *L. crispatus* presents antagonistic activity resulting from the production of other antimicrobial compounds, including, for example, bacteriocins (Ojala et al., 2014). Bacteriocins are ribosomal synthesized antimicrobial peptides produced by various bacterial species, whose main function is to kill and / or inhibit the growth of other

bacteria. These antimicrobial compounds act through the formation of pores in the cytoplasmic membrane of the target bacteria, which, by altering the permeability of the membrane, increase the flow of transmembrane ions, causing a decrease in intracellular pH, inhibiting enzymatic processes, leading to cell death (Mokoena, 2017).

Based on their primary structures, molecular weights, post-translational modifications, and genetic characteristics, bacteriocins are categorized into three main classes: (i) Class I, also named lantibiotics, includes small sized (<5 kDa), cationic and hydrophobic peptides, such as nisin and lactocin); (ii) Class II includes unmodified, small size (<10 kDa), heat-stable and cationic hydrophobic peptides. Depending on the mode of action, this class is subdivided into Class IIa (such as pediocin PA1 and leukocidin) and Class IIb (such as plantaricin A and enterocin X). Class III includes large (>30 kDa), hydrophilic, and heat-labile peptides, such as helveticin J and enterolysin (Mokoena, 2017).

In *L. crispatus* genomes, several genes encoding bacteriocins were previously identified using the BAGEL (BACTERIOCIN GENOME MINING TOOL) web server. Almeida et al. (2021) identified genes encoding the bacteriocins helveticin J, enterolysin A, and penocin A, and other genes associated with bacteriocins in their study of the genome of four *L. crispatus* strains (CRI4 CRI8 CRI10 CRI17). In other strains of *L. crispatus* (VMC1, VMC2, VMC3, VMC4, VMC5, VMC6, VMC7, VMC8), in addition to multiple genes encoding putative enterolysin A and helveticin-J, genes encoding for thermophilin A, durancin Q, coagulins, and staphylococin C55 β bacteriocins have also been identified (Abdelmaksoud et al., 2016). Thus, the production of these antimicrobial compounds appears to be a promising strategy for bacterial vaginosis treatment and could contribute to urogenital health.

Other studies have suggested that biosurfactants produced by lactobacilli strains have beneficial effects, such as nutrient transport, bacterial–host interactions, antimicrobial activity, and inhibition of biofilm formation and adherence (Satpute et al., 2016). These compounds have great biotechnological potential for industry as they are not considered toxic for humans and are easily degraded in the environment. In this context, De Gregorio et al. (2020) investigated the role of biosurfactants from *L. crispatus*; they found protective activity against *Candida* spp. *in vivo* and *in vitro*.

Lactic acid synthesis

Lactic acid is the main substance produced by lactobacilli; it helps protect against vaginal infections. The main mechanism of protective action relies on pH reduction to under 4.5, reaching values of less than 3.5 when the vaginal microbiota is dominated by *Lactobacillus* spp., protecting against a wide variety of non-indigenous pathogens (Smith and Ravel, 2017). Lactic acid is produced by *Lactobacillus* spp. in two isomeric forms, D- and L-lactic acid; the proportion of their relative production is species and strain dependent (Abdelmaksoud et al., 2016). Species such as *L. iners* do not have genes that produce D-lactic acid and produce lower concentrations of L-lactic acid when compared to *L. crispatus* and *L. gasseri*, while *L. jensenii* is only able to produce the D form (Smith and Ravel, 2017).

L. crispatus is a potent producer of lactic acid and other bacterial inhibitor compounds; consequently, when the vaginal microbiome is highly colonized by *L. crispatus*, it is less likely to develop bacterial vaginosis (Abdelmaksoud et al., 2016). *L.*

crispatus encodes two genes for the L form of the enzyme and one for the D form (Almeida et al., 2021).

In a cohort study of the vaginal microbiome of 255 pre-menopausal nonpregnant women, 20% presented an *L. crispatus* dominant microbiome, and these women were less likely to be colonized by *Candida* spp. (Tortelli et al., 2020). This study also showed that, *in vitro*, an *L. crispatus* cell-free supernatant has a pH of around 4.0 and high levels of lactic acid, being able to inhibit *C. albicans* growth. Another study isolated 135 *Lactobacillus* strains from the vaginal microbiome of reproductive women; among these, 56% were *L. crispatus* (Hütt et al., 2016). These strains were tested against *E. coli*, *C. glabrata* and *G. vaginalis*, using a modified agar spot method; *L. crispatus* had the highest activity against all three species when compared to the other *Lactobacillus* species (Hütt et al., 2016). These results show that lactobacilli can help maintain vaginal health, especially if the vaginal microbiome is *L. crispatus* dominated.

Insights concerning genomic islands

Genomic islands (GEIs) are key elements in the bacterial accessory genome as they can facilitate the acquisition of novel genes, conferring a fitness benefit in specific habitats (Soares et al., 2016). Investigating GEI regions in *L. crispatus* strains provides important insights into the evolution, lifestyle adaptation, and metabolic diversity of this species, which may be useful for screening probiotic strains.

The four genomes of *L. crispatus* strains previously isolated by Almeida et al. (2021) present nine putative GEIs in the strains CRI4 and CRI17, and 14 in CRI8 and CRI10. Each GEI has an approximate size of 15,477 bp; together they correspond to 7% of the total genome size of *L. crispatus*. Most gene content in the GEIs is related to phage integration, bacterial metabolism, and survival, such as type II-A CRISPR-associated proteins and thioredoxin family proteins.

Potential applications for vaginal health

Metagenomics studies investigating vaginal microbiota related to a healthy status might reveal crucial details about the protective role of *Lactobacillus* spp., especially *L. crispatus*. It has been suggested that the presence of this bacterium in the vaginal environment is less associated with potential pathogenic species usually found in polymicrobial infections. This ability to maintain a balanced microbial community was confirmed *in vitro*; *L. crispatus* strains were able to prevent the growth of various pathogens (Mancabelli et al., 2021).

Some species found in the vaginal microbiota, such as *Gardnerella* spp. and other lactobacilli, including *L. iners*, secrete toxins known as vaginolysin and inerolysin (Pleckaityte M, 2020). *L. crispatus* has been shown to decrease the expression of vaginolysins produced by *Gardnerella* spp. (Castro et al., 2018), revealing a protective role. These authors suggest the toxins could serve as biomarkers for bacterial vaginosis diagnostics.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abdelmaksoud AA, Koparde VN, Sheth NU, Serrano MG, et al. (2016). Comparison of *Lactobacillus crispatus* isolates from *Lactobacillus*-dominated vaginal microbiomes with isolates from microbiomes containing bacterial vaginosis-associated bacteria. *Microbiology*. 162: 466-475. doi: 10.1099/mic.0.000238.
- Almeida MO de, Carvalho R, Aburjaile FF, Miranda FM, et al. (2021). Characterization of the first vaginal *Lactobacillus crispatus* genomes isolated in Brazil. *Peer. J.* 9: e11079. doi: 10.7717/peerj.11079.
- Castro J, Martins AP, Rodrigues ME and Cerca N (2018). *Lactobacillus crispatus* represses vaginolysin expression by BV associated *Gardnerella vaginalis* and reduces cell cytotoxicity. *Anaerobe*. 50: 60-63. doi: 10.1016/j.anaerobe.2018.01.014.
- Castro J, Machado D and Cerca N (2019). Unveiling the role of *Gardnerella vaginalis* in polymicrobial Bacterial Vaginosis biofilms: the impact of other vaginal pathogens living as neighbors. *ISME J.* 13: 1306-1317. doi: 10.1038/s41396-018-0337-0.
- De Gregorio PR, Parolin C, Abruzzo A, Luppi B, et al. (2020). Biosurfactant from vaginal *Lactobacillus crispatus* BC1 as a promising agent to interfere with *Candida* adhesion. *Microb. Cell Fact.* 19: 1-16. doi: 10.1186/s12934-020-01390-5.
- Hertzberger R, Arents J, Dekker HL, Pridmore RD, et al. (2014). H₂O₂ Production in Species of the *Lactobacillus acidophilus* Group: a Central Role for a Novel NADH-Dependent Flavin Reductase. *Appl. Environ. Microbiol.* 80: 2229-2239. doi: 10.1128/AEM.04272-13.
- Hütt P, Lapp E, Štšepetova J, Smidt I, et al. (2016). Characterisation of probiotic properties in human vaginal lactobacilli strains. *Microb. Ecol. Health Dis.* 27: 30484. doi: 10.3402/mehd.v27.30484.
- Pleckaityte M (2020). Cholesterol-Dependent Cytolysins Produced by Vaginal Bacteria: Certainties and Controversies. *Front Cell Infect Microbiol.* 9: 452. doi: 10.3389/fcimb.2019.00452.
- Mancabelli L, Mancino W, Lugli GA, Milani C, et al. (2021). Comparative genome analyses of *Lactobacillus crispatus* isolated from different ecological niches reveal an environmental adaptation of this species to the human vaginal environment. *Appl Environ Microbiol.* 12: 02899. doi: 10.1128/AEM.02899-20
- Matu MN, Orinda GO, Njagi ENM, Cohen CR, et al. (2010). In vitro inhibitory activity of human vaginal lactobacilli against pathogenic bacteria associated with bacterial vaginosis in Kenyan women. *Anaerobe*. 16: 210-215. doi: 10.1016/j.anaerobe.2009.11.002.
- Mokoena MP (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. *Molecules*. 22: 1255. doi: 10.3390/molecules22081255.
- Ojala T, Kankainen M, Castro J, Cerca N, et al. (2014). Comparative genomics of *Lactobacillus crispatus* suggests novel mechanisms for the competitive exclusion of *Gardnerella vaginalis*. *BMC Genomics*. 15: 1070. doi: 10.1186/1471-2164-15-1070.
- Satpute SK, Kulkarni GR, Banpurkar AG, Banat IM, et al. (2016). Biosurfactant/s from *Lactobacilli* species: properties, chSatpute, Surekha K., et al. "Biosurfactant/s from Lactobacilli species: Properties, challenges and potential biomedical applications. *J. Basic Microbiol.* 56(11): 1140-1158. doi: <https://doi.org/10.1002/jobm.201600143>.
- Smith SB and Ravel J (2017). The vaginal microbiota, host defence and reproductive physiology. *J. Physiol.* 595: 451-463. doi: 10.1113/JP271694.
- Soares SC, Geyik H, Ramos RTJ, de Sá PHCG, et al. (2016). GIPSY: Genomic island prediction software. *J. Biotechnol.* 232: 2-11. doi: 10.1016/j.jbiotec.2015.09.008.
- St Amant DC, Valentin-Bon IE and Jerse AE (2002) Inhibition of *Neisseria gonorrhoeae* by *Lactobacillus* species that are commonly isolated from the female genital tract. *Infect Immun* 70:7169–7171. doi: 10.1128/iai.70.12.7169-7171.2002
- Tachedjian G, O'Hanlon DE and Ravel J (2018). The implausible "in vivo" role of hydrogen peroxide as an antimicrobial factor produced by vaginal microbiota. *Microbiome*. 6: 29. doi: 10.1186/s40168-018-0418-3.
- Tortelli BA, Lewis WG, Allsworth JE, Member-Meneh N, et al. (2020). Associations between the vaginal microbiome and *Candida* colonization in women of reproductive age. *Am. J. Obstet. Gynecol.* 222:471.e1-471.e9. doi: 10.1016/j.ajog.2019.10.008.
- Zheng HY, Alcorn TM and Cohen MS (1994). Effects of H₂O₂-producing lactobacilli on *Neisseria gonorrhoeae* growth and catalase activity. *J. Infect. Dis.* 170: 1209-1215. doi: 10.1093/infdis/170.5.1209.