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Lactobacillus crispatus protects against bacterial vaginosis

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ABSTRACT. In medicine, the 20th century was marked by one of the most important revolutions in infectious-disease management, the discovery and increasing use of antibiotics. However, their indiscriminate use has led to the emergence of multidrug-resistant (MDR) bacteria. Drug resistance and other factors, such as the production of bacterial biofilms, have resulted in high recurrence rates of bacterial diseases. Bacterial vaginosis (BV) syndrome is the most prevalent vaginal condition in women of reproductive age,

leading to considerable discomfort. BV can be a consequence of gynecological and obstetric complications, as well as sexually transmitted diseases. Given the decrease in efficiency of antibiotic therapy and high rates of recurrence, probiotics have become promising alternatives for both prevention and treatment of BV, or as an adjuvant to conventional therapy. Currently, *Lactobacillus* species are the most extensively studied for use as probiotics. Probiotics act through stimulation of the host immune system, competitive exclusion and antimicrobial activity; the latter involves production of substances such as lactic acid, hydrogen peroxide and bacteriocins. *Lactobacillus crispatus* is considered to be a biomarker of a healthy vaginal tract and is indicated for a probiotic approach to maintaining and restoring of a healthy vaginal ecosystem. Some *L. crispatus* probiotic strains are already commercially available with encouraging results; however, control of BV syndrome still presents many challenges.

Key words: Probiotic; *Lactobacillus crispatus*; Bacterial Vaginosis; Antibiotic failure; Competitive exclusion

INTRODUCTION

Antibiotics kill or inhibit the growth of microorganisms, especially bacteria. These drugs have been widely used in the prevention and treatment of bacterial infection (Gould, 2016). Since the discovery of the first antibiotic - penicillin in 1928, the increasing indiscriminate use of antibiotics has led to the spread and accumulation of antibiotic resistance genes. In recent years, inappropriate prescribing and use of these drugs has lead to bacterial resistance, especially the multidrug-resistant (MDR) bacteria (Navon-Venezia et al., 2017; World Health Organization, 2018). The high recurrence rates of bacterial diseases due to antibiotic resistance and the production of bacterial biofilms (Senok et al., 2009) has motivated current efforts into discovering and developing new alternatives to the use of antibiotics. Within this context, probiotics are a promising new approach both for the prevention and treatment of some diseases (Ghosh et al., 2019).

The term “probiotics” refers to microorganisms that confer a health benefit to the host when administered in adequate amounts (Hill et al., 2014). The safety characteristics or Generally Recognized as Safe (GRAS) status ensures that the probiotic is unable to transfer resistance to antibiotics and pathogenicity and toxicity factors. The functional characteristics are related to successful administration of the probiotic, stable permanence in the host and beneficial health effects. Probiotic bacteria can stimulate the immune system or act to suppress pathogens. However, these potential beneficial effects must be verifiable in the host (reviewed by Kechagia et al., 2013; reviewed by Plaza-Díaz et al., 2018).

Lactic acid bacteria (LAB) are the most widely studied group of probiotics. Among these, lactobacilli species have attracted considerable attention. They can play a dual role in both production/conservation of foods and health promotion (Sanders et al., 2019). *Lactobacillus* is an abundant and heterogeneous Gram-positive bacterial genus. Various species of this genus occupy a wide variety of carbohydrate-rich niches, are found in

diverse environments, including plants, animals and fermented foods, and are part of the microbiota of the oral and nasal cavity, gastrointestinal tract and urogenital tract of vertebrates (George et al., 2018).

The vaginal microbiome of healthy women generally has lactobacilli as the predominant bacteria. Analysis of the healthy vaginal microbiome by sequencing of the bacterial 16S rRNA gene revealed five community state types (CSTs), four of which were dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners* or *Lactobacillus jensenii*; CSTs with the latter species had lower proportions of lactic acid bacteria and higher proportions of strictly anaerobic organisms (Ravel et al., 2011).

Imbalances in the healthy vaginal ecosystem can result in increased growth of anaerobic bacteria, leading to bacterial vaginosis (BV) syndrome (Eschenbach et al., 1988). There are still many controversies concerning BV syndrome because its etiology and evolution are not entirely clear. While some women are asymptomatic, others experience great discomfort. Consequently, various nomenclatures have been assigned to BV. Several studies suggest a relationship between BV and gynecological and obstetric complications and diseases (Reid, 2018). *Gardnerella vaginalis* is an opportunistic anaerobic vaginal pathogen that often exhibits a symbiotic relationship with other anaerobes (Castro et al., 2017). Most BVs are characterized by bacterial biofilm formation, in which the usually prevalent species is *G. vaginalis* (Marrazzo et al., 2008). Antibiotic treatment often fails to fully eradicate the BV pathogenic biofilm. Consequently, there is a high rate of inefficiency in antibiotic treatment, probably increasing bacterial resistance and recurrence of BV (Swidsinski et al., 2005; Kim and Park, 2017).

L. crispatus is the most prevalent species of lactobacilli present in the vaginal ecosystem; it is considered to be a protective agent and biomarker of a healthy vaginal tract (Lepargneur, 2016). Here, we review current knowledge on probiotics with a focus on *L. crispatus* in the prevention and treatment of BV. We discuss how competitive exclusion, bacteriostatic and bactericidal factors allow *L. crispatus* to compete with other microorganisms and occupy an ecologically stable vaginal niche.

Bacterial vaginosis

Etiology and evolution of bacterial vaginosis

Bacterial vaginosis is one of the most common causes of genital discomfort in women (Kim and Park, 2017; Reid, 2018). Previously, the term bacterial vaginosis was used to describe a syndrome characterized by the presence of malodorous discharge, without apparent inflammation, resulting from complex alterations in the vaginal microbiota (Eschenbach et al., 1988). With traditional diagnostic methods, many women are classified as positive for BV even though they are asymptomatic. Only 50-60% of women diagnosed with BV report symptoms such as a malodorous “fishy” vaginal discharge (Senok et al., 2009). In the review of Mitchell and Marrazzo (2014), they concluded “BV is not the same microbiologic syndrome in all women”, thus also describing BV as a “syndrome” (Mitchell and Marrazzo, 2014). Due to a lack of consensus in the scientific community, Reid (2018) questioned whether BV is a disease. BV has been described as “a disease, a disorder, a vaginal inflammation, an infection, a microbial dysbiosis, a condition, and in some women, a normal situation”.

Some bacteria that cause BV may be present as commensals in the vaginal environment. For reasons not yet fully elucidated, this vaginal dysbiosis occurs as a result of perturbation of the healthy vaginal microbiome (Reid, 2018). BV is characterized by an increased bacterial diversity due to a decrease in beneficial bacteria and a concomitant over proliferation of various other bacteria. Consequently, the commensal microbiota with a prevalence of lactobacilli is replaced by various anaerobic genera. *G. vaginalis* is the most frequent detected BV-causing anaerobic bacterium, being present in approximately 95% of all cases (Marrazzo et al., 2008). Other anaerobic bacteria have also been associated with BV, including *Prevotella* spp., *Porphyromonas* spp., *Bacteroides* spp., *Peptostreptococcus* spp., *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mobiluncus* spp., *Fusobacterium* spp., and *Atopobium vaginae* (Marrazzo, 2004; Vaneechoutte et al., 2004; Fredricks and Marrazzo, 2005; Javed et al., 2019). A relationship between prevalence of *Prevotella* spp. and host genetics has been suggested. This bacterial genus has been strongly associated with menopause, obesity, HPV infection and BV (Si et al., 2017).

Most cases of BV are characterized by the adherence of a bacterial biofilm to the vaginal epithelium (Swidsinski et al., 2005). *G. vaginalis* develops in one of two phenotypes: planktonic or sessile. These two lifestyles exhibit different patterns of gene transcription and growth rate (di Luca et al., 2014; Castro et al., 2017). While the planktonic phenotype allows for rapid growth under favorable conditions, the sessile phenotype includes biofilm formation, which contributes significantly to treatment failure and high BV recurrence rates (Senok et al., 2009; Ravel et al., 2011).

Briefly, the biofilm structure consists of a multitude of microbial cells associated with a surface and enclosed in a matrix consisting of extracellular polymeric substances (EPS) that they produce. This EPS matrix contributes to the antimicrobial-resistance properties of biofilms (Donlan, 2002; Senok et al., 2009). Multi-species biofilm formation can be complex and depends upon interactions between the species involved. As *G. vaginalis* is usually the main component of the BV biofilm, this bacterium possibly initiates biofilm formation and allows the successive adhesion and proliferation of other species. It has been found that *G. vaginalis* growth increases in the presence of other species (Machado et al., 2013). The biofilm forming microorganisms display a series of specific mechanisms for the construction of the biofilm, with initial attachment to a surface, development of a dynamic community structure with formation of a characteristic microbiome, and detachment and dissolution of the biofilm for rapid growth of the microorganisms under favorable conditions. The main line of communication between microbial cells in a community, such as a biofilm, is through quorum sensing (Parsek and Greenberg, 2005; Senok et al., 2009). Thus, under adverse conditions, a biofilm lifestyle provides an ecological advantage over a planktonic phenotype in the vaginal environment (Donlan, 2002; Senok et al., 2009; Machado et al., 2013).

With the advent and refinement of molecular techniques, hundreds of bacteria have been identified in vaginal microbiota. Recent studies in this context have been shown that BV is definitely not a disease as diagnosed clinically or based on normal laboratory analyses (Reid, 2018). The vaginal microbiota is a dynamic community that seems to change in response to hormonal fluctuations in the female body, race/ethnicity, use of intrauterine devices, new sexual partners or multiple sexual partners, vaginal douching, and low socioeconomic level, among other influences. The composition of the vaginal microbiota and the dominant microbial species vary from woman to woman. In addition,

recent reviews of studies on cellular immune parameters suggest that inflammation occurs in some BV-positive women (Mitchell and Marrazzo, 2014; Borgdorff et al., 2016; Reid, 2018). Therefore, it appears that each woman can have a specific vaginal microbiota pattern in response to different situations and unique adversities, justifying the differences in the BV patterns observed. Considering that many cases are asymptomatic, other cases do not respond to antibiotics and that even in cases that are responsive, the antibiotic may not prevent recurrence (Kim and Park, 2017; Reid, 2018), many questions about the etiology and evolution of BV syndrome persist.

Complications related to BV

One of the main concerns regarding BV is other diseases or complications that may be related to this syndrome. BV can be associated with various potentially severe gynecological and obstetric complications and sequels, and with sexually transmitted infections (STIs). Studies suggest that only the presence of BV-associated bacteria is not sufficient to result in other diseases or complications (Mitchell and Marrazzo, 2014; Borgdorff et al., 2016), which raises the hypothesis of the need for an inflammatory process.

In the case of infertility, an association between BV, pelvic inflammatory disease and tubal factor infertility was suggested, but BV could not be distinguished as a cause or effect (Wilson et al., 2002). In women undergoing *in vitro* fertilization, BV can result in lower implantation rates and increased rates of early pregnancy loss (Eckert et al., 2003; Verstraelen and Senok, 2005). Pregnant women with BV have a higher risk of adverse outcomes, such as late miscarriage, chorioamnionitis, premature rupture of membranes, preterm birth and *postpartum* endometritis (Marrazzo, 2004; DiGiulio et al., 2008; Peelen et al., 2019). In the case of preterm birth, bacteria in the amniotic fluid without inflammation may be benign (Cobo et al., 2014). Consequently, *Lactobacillus*-dominated vaginal ecosystems appear to be beneficial for various obstetric outcomes.

Growing evidence shows that BV is a risk factor for STIs. There is a correlation between the absence of vaginal lactobacilli, BV and various common STIs caused by bacteria, protozoa and viruses (Martin et al., 1999; Cherpes et al., 2003; Peipert et al., 2008; Brotman et al., 2010). Studies have shown correlations between BV and STIs caused by the bacteria *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitalium*, the protozoan *Trichomonas vaginalis*, and viruses such as the human immunodeficiency virus (HIV), human papilloma virus (HPV), and herpes simplex virus type 2 (HSV-2) (Brotman, 2011; Anahtar et al., 2018).

In particular, concerning HIV, evidence suggests that both women and men are at increased risk for acquisition of the virus as a consequence of BV or in the absence of vaginal lactobacilli (Adimora et al., 2008; Cohen et al., 2012; Gosmann et al., 2017; Eastment and McClelland, 2018). Apparently, BV can elevate these risks in several ways, including upregulation of relevant T-cell populations, reducing protective factors, increasing inflammatory factors, increasing viral replication and vaginal shedding of HIV. Since the etiology of all such relationships involving BV, lactobacilli and STIs is still potentially misinterpreted by common risk factors associated with STIs, further studies on BV-related cellular and immunological parameters are needed (Brotman, 2011; Anahtar et al., 2018; Marrazzo, 2018).

Conventional treatment of BV

Current guidelines for treatment of BV have been based on oral or intravaginal administration of metronidazole and clindamycin. Other antibiotics that have been used include nitroimidazole, secnidazole, and tinidazole, depending on the bacterial specificity required (Marrazzo, 2004; Javed et al., 2019). In addition to antibiotics, other drugs used in the attempt to treat BV include cellulose sulfate, polystyrene sulfinate and policarbophilcarbopol acidic vaginal gel (Simoes et al., 2002; Javed et al., 2019). Although antibiotics contribute to a decrease in the bacterial burden that causes vaginal infections, their use is debatable due to adverse effects, recurrences, antibiotic resistance and lack of selectivity, since antibiotics also reduce or eliminate beneficial bacteria. Up to 10-15% of patients undergoing administration of metronidazole or clindamycin fail to respond to initial antimicrobial therapy (Kim and Park, 2017). Additionally, BV recurrence rates among patients who showed an initial response to antibiotic therapy also remain high, reaching up to 80%, necessitating repeated administration of antibiotics. The need for long-term or repeated antibiotic exposure increases the risk of emergence of resistant strains, alteration of microbiota, and possible persistence of BV-associated pathogens (Kim and Park, 2017).

Probiotic therapy for BV

Due to the particularities of the vaginal microbiota in each woman and the high recurrence rates associated with antibiotic therapy against BV, many questions about the etiology, evolution and, consequently, about the treatment of this condition remain (Reid, 2018). Probiotic *Lactobacillus* strains have been shown to be the most promising alternatives for both prevention and treatment of BV, which may or not be used synergistically with the administration of antibiotics (Homayouni et al., 2014; Javed et al., 2019).

Benefits of probiotic organisms

There is increasing evidence for the health benefits of probiotics, including enhancement of the immune response (Khalesi et al., 2018), a protective role against allergy (Sharma and Im, 2018; Szari and Quinn, 2019), removal of chemical contaminants and ability to reduce the toxicity of contaminants (Feng et al., 2018; Wochner et al., 2018; Wang et al., 2019), improving intestinal health by modulating the number and diversity of beneficial gut microbiota, and reducing symptoms associated with various gastrointestinal disorders (Ferrario et al., 2014; Ford et al., 2014; Feng et al., 2018; Irwin et al., 2018; Vasquez et al., 2019), hepatoprotective effects (Meng et al., 2018), cardiovascular disease prevention by improving blood cholesterol levels and blood lipid profile, reducing blood pressure and hypertension (Guo et al., 2011; Khalesi et al., 2014; Sun and Buys, 2015; Vasquez et al., 2019), improving blood glucose tolerance and diabetes control (Sun and Buys, 2016; Plows et al., 2019), improved mental health (Inserra et al., 2018; Bruce-Keller et al., 2018; Dutta et al., 2019), and cancer prevention (Nazir et al., 2018; Scott et al., 2018; Ma et al., 2019). However, in order to improve the probability of these beneficial effects, probiotic candidate strains should be submitted to selection assays, which can be crucial to promulgate the therapeutic effect in the host.

Criteria for selection of probiotic strains

Probiotic strains are selected for potential application based on particular physiological and functional properties, some of which may be determined *in vitro* (Carmo et al., 2017). Many studies suggest that probiotic effects are strain-specific. A beneficial effect attributed to one strain may not be found in another, even though it belongs to the same species. Therefore, in order to establish their suitability and performance for industrial application, strain characterization is required (Ibnou-Zekri et al., 2003; Kechagia et al., 2013; Abdelmaksoud et al., 2016).

Several aspects are relevant in the selection of probiotic microorganisms, including safety, functional and technological characteristics. Safety is mainly related to concerns about pathogenicity and disease, including not carrying transmissible antibiotic resistance genes. Therefore, safe microorganisms cannot have a history of pathogenicity or an association with diseases in the host, and they cannot disseminate genes related to antibiotic resistance through horizontal gene transfer (Saarela et al., 2000).

It is expected that a potential probiotic strain displays certain desirable properties and functional aspects. The functional aspects are mainly related to survival in the host and beneficial effects provided. When administered orally, a probiotic should have the ability to survive under adverse conditions suffered throughout the gastrointestinal tract. Once inside the host, stable permanence is related to the ability to adhere to mucosal and epithelial surfaces. Functional aspects related to these adaptive attributes that are currently determined by *in vitro* tests are listed in Table 1 (Saarela et al., 2000; Kechagia et al., 2013).

Table 1. Functional aspects relevant to the selection of probiotic microorganisms.

Functional aspects	Purpose
Acid and bile tolerance	Probiotic survival in oral administration
Bile salt hydrolase activity	Resistance to action of bile salts in oral administration
Adhesion to mucosal and epithelial surfaces	Effective colonization, which is an important feature for health benefits such as successful immune modulation and competitive exclusion of pathogens
Antimicrobial activity against pathogenic bacteria such as production of lactic acid, hydrogen peroxide and bacteriocins	Effective colonization and health benefits that avoid complications and diseases caused by pathogenic microorganisms

Technological aspects are mainly related to probiotic production and processing for industrial application. These include phage resistance, viability during processing and stability in production and during storage (Saarela et al., 2000). Viability is, by definition, a prerequisite for probiotic functionality; however, certain studies (Salminen et al., 1999; Lahtinen, 2012) have demonstrated that viability is not necessary for all probiotic effects. Thus, in some cases, good viability during storage can be dispensed with if an optimal growth rate has been obtained during the initial production steps. In addition, probiotic encapsulation technology can ensure viability and stability (Salminen et al., 1999; Saarela et al., 2000; Lahtinen, 2012; Kechagia et al., 2013).

Bacterial strains of *Bifidobacterium* and *Lactobacillus* have a long history of safe and effective use as probiotics. The most commonly used yeast is *Saccharomyces boulardii* (Sanders et al., 2019). Other genera have also been marketed as probiotics, although

concerns remain regarding the safe use of these organisms for this purpose (Eaton and Gasson, 2001; Holzapfel et al., 2001; Ishibashi and Yamazaki, 2001; Balakrishnan and Floch, 2012; Sanders et al., 2019).

The use of lactobacilli in the treatment of Bacterial Vaginosis

Species of *Lactobacillus* vary in their stability and protective ability in the vaginal environment (Tamrakar et al., 2007; Verstraelen et al., 2009; Abdelmaksoud et al., 2016). Stability is related to permanence of the lactobacilli in the vaginal environment, with their not being easily displaced, even in the face of various adversities, such as hormonal changes, sexual activity and exposure to semen, altered nutrient sources, transient fluctuations in pH, and exposure to non-resident bacterial species. Meanwhile, the protective ability reflects the competitive features of the lactobacilli, which prevent vaginal colonization by BV-associated anaerobes (Abdelmaksoud et al., 2016).

Probiotic lactobacilli have been administered through oral and vaginal routes to attempt BV prevention and treatment. As reviewed by Homayouni et al. (2014) and Javed et al. (2019), single and multiple strains have been evaluated in randomized clinical trials alone and with antibiotic therapy. However, it is still unclear which route is most efficient for each purpose. Since lactobacilli colonizing the rectum may be a reservoir for vaginal lactobacilli (Antonio et al., 2005), the oral route has been suggested as more practical and could both prevent BV and provide other health benefits for the consumer. On the other hand, the vaginal route allows the introduction of probiotic bacteria directly into the vagina through probiotic capsules, creams, gels or tampons filled with freeze-dried lactobacilli. *Lactobacillus* strains that have been investigated for BV prevention and treatment include *L. reuteri*, *L. fermentum*, *L. gasseri*, *L. rhamnosus*, *L. brevis*, *L. acidophilus*, *L. plantarum* and *L.crispatus* (Reid et al., 2001, 2003; Homayouni et al., 2014; De Alberti et al., 2015; Recine et al., 2016; Verdenelli et al., 2016; Javed et al., 2019).

Most relevant clinical trials have shown that oral or intravaginal administration of *L. acidophilus* strains, or intravaginal administration of *L. fermentum* RC-14 combined with *L. rhamnosus* GR-1 are able to increase the numbers of vaginal lactobacilli and restore a healthy vaginal microbiota (Homayouni et al., 2014). Although these strains have been extensively investigated as probiotics to help prevent urogenital infections, the use of *Lactobacillus* species that are naturally more prevalent in the vagina of healthy women has been a more promising option for the vaginal probiotic approach (Lepargneur, 2016; Javed et al., 2019). The use of these species could ensure greater success in effective and lasting colonization of lactobacilli in the vaginal environment, which is a determining factor for the prevention, treatment and reduction of BV recurrence rates. *L. crispatus* is the most prevalent species of lactobacilli present in the healthy vaginal ecosystem (Ravel et al., 2011; Lepargneur, 2016).

Lactobacillus crispatus as a probiotic against Bacterial Vaginosis

Lactobacillus crispatus is one of the most frequent bacteria in the human gastrointestinal and genitourinary microbiota that provides benefits to the host. This bacterium can comprise more than 80% of all bacteria in the healthy vagina (Ravel et al., 2011) and is considered to be one of the most active, stable and protective species in this

environment (Macklaim et al., 2013; Abdelmaksoud et al., 2016). Verstraelen et al. (2009) reported a five-fold decreased risk for developing BV in pregnant women colonized with *L. crispatus*. A longitudinal analysis of the vaginal microbiota of 100 Caucasian pregnant women suggested that *L. crispatus* promotes stability in the healthy vagina, whereas *L. gasseri* and/or *L. iners* are more conducive to vaginal dysbiosis (Verstraelen et al., 2009). In a study by Gajer et al. (2012), the stability and protective role of *L. crispatus* was also observed. The temporal dynamics of the composition of vaginal bacterial communities in 32 healthy reproductive-age women revealed that some communities change markedly over short time periods. Meanwhile, other communities are relatively stable, including communities dominated by *L. crispatus* (Gajer et al., 2012).

An inverse association between *L. crispatus* and *G. vaginalis* has been reported; the latter is one of the main bacteria associated with BV (Fredricks et al., 2007; Srinivasan et al., 2012; Shipitsyna et al., 2013). However, the mechanisms involved in the protective role of *L. crispatus* are not entirely clear. It is known that the roles of protective or pathogenic vaginal bacterial species, in health or disease, can be influenced by genetic differences between strains (Ojala et al., 2014; Abdelmaksoud et al., 2016; Borgdorff et al., 2016; France et al., 2016; Salas et al., 2016).

The *L. crispatus* genome is about 2.0–2.7 Mb in size (Human et al., 2010; Ojala et al., 2010) and is highly variable, with the core genome making up only ~60% (Ojala et al., 2014; Abdelmaksoud et al., 2016). Compared to other more frequent vaginal lactobacilli, *L. crispatus* has the largest genome size (Mendes-Soares et al., 2014). However, its genome is still smaller than the genome of other *Lactobacillus* species that are non-host associated (France et al., 2016). These characteristics may help explain the prevalence of *L. crispatus* in the vaginal environment and its specialization to occupy the vaginal niche.

In silico insights about the role of *L. crispatus* in BV

A decrease of *L. crispatus* populations during the evolution of BV has been proposed. Bacteriophages are natural inhibitors of bacteria and temperate phages are common in vaginal lactobacilli (Pavlova et al., 1997; Kilic et al., 2001; Damelin et al., 2011). *In silico* analyses allow us to identify prophage clusters, including defective prophages that are retained in the bacterial chromosome, which are unable to excise, replicate or lyse the bacteria. In a comparative genomic study of 10 strains, Ojala et al. (2014) identified 31 prophage-like regions, comprising 1,636 CDSs and accounting for more than a fifth of the ortholog groups in *L. crispatus*. Curiously, the prophage-like clusters were enriched in all vaginal isolates of *L. crispatus*, but they were not found in the chicken isolate ST1 (Ojala et al., 2014).

A high degree of lysogeny (77%) was observed for vaginal strains of *L. crispatus* by Damelin et al. (2011) and confirmed by Ojala et al. through *in silico* analyses (Damelin et al., 2011; Ojala et al., 2014). This suggests that transduction is an important mechanism for genome evolution in these bacteria. Abdelmaksoud et al. (2016) performed *in vitro* analyses to determine whether there is a correlation between phage inhibition of *L. crispatus* and BV development. However, their analyses did not show a strong correlation between phage induction and bacterial lysis. Moreover, no correlation was observed between phage inhibition of *L. crispatus* and BV development since the degree of bacterial lysis did not differ between the group of strains from lactobacilli-dominated microbiomes and strains

from microbiomes containing BV-associated bacteria (Abdelmaksoud et al., 2016). Phages may influence the stability of *L. crispatus*, but differences in stability are not simply explained by the presence or absence of inducible phages. Thus, further studies should be carried out to investigate the complex interactions between the *L. crispatus* strains, their environment and the possibility of phage induction (Ojala et al., 2014; Abdelmaksoud et al., 2016).

L. crispatus could contribute to the maintenance or restoration of urogenital health through competitive exclusion and antimicrobial activity. In a probiotic approach, there should be a specific focus on the molecular mechanisms involved in host-probiotic and probiotic-pathogen interactions. These mechanisms involve genes implicated in the production of adhesion-associated compounds, exopolysaccharides (EPS), surface-layer (S-layer) proteins and antimicrobial substances (Ojala et al., 2014).

Adherence and competitive exclusion

Adhesion to host tissue has been considered a feature that enhances probiotic activity (Carmo et al., 2017; do Carmo et al., 2018). In addition to being a prerequisite for the effective colonization of the vaginal environment, adhesion of *L. crispatus* stimulates the host's immune system and has antagonistic activity against pathogenic microorganisms through competitive exclusion (Boris and Barbés, 2000; Ojala et al., 2014). In terms of bacterial pathogenesis, adherence to host cells plays a crucial role in biofilm forming bacteria and cell surface polysaccharides have an established role as virulence factors (Hardy et al., 2017). In contrast, there is a lack of information about biofilm formation and the probiotic feature of competitive exclusion performed by lactobacilli. Both biosynthesis and the biological function of cell surface polysaccharides are poorly reported in probiotic *Lactobacillus* spp. and their ability to inhibit adhesion and biofilm formation of other species also has not yet been fully elucidated (Salas et al., 2016).

Analyzing the *L. crispatus* proteome, Ojala et al. (2014) identified 103 putative adhesins related to the ability to colonize and interact with the host. In addition, they found ~30 putative S-layer proteins encoding genes that could potentially contribute to bacterial adhesion and competitive exclusion (Ojala et al., 2014). Some studies have reported that S-layer proteins are related to inhibition of adhesion, competitive exclusion and synergistic action against various microorganisms in non-vaginal environments (Horie et al., 2002; Chen et al., 2007; Sun et al., 2017; do Carmo et al., 2018).

It is well known that BV can form a multi-species biofilm in which *G. vaginalis* is the most common dominant bacterial strain (Swidsinski et al., 2005). Curiously, a highly variable genome region appears to be associated with EPS biosynthesis in *L. crispatus*. Ojala et al. reported that the EPS gene cluster is comprised of 37 EPS biosynthesis genes, five of which appear to be always present in the operon of all strains. The EPS genetic variations may be related to adhesion, biofilm formation, and competitive exclusion of pathogens (Ojala et al., 2014). It was reported that *L. crispatus* strains that are producers of high levels of EPS can have probiotic potential, including cholesterol-reducing features (Anandharaj et al., 2015; Khalil et al., 2018). Furthermore, Donnarumma et al. (2014) observed that a specific *L. crispatus* EPS presents structural features and similarity to exopolysaccharides produced by pathogenic strains; they also found that *L. crispatus* cells strongly reduced adhesion of a pathogenic yeast strain. Using confocal laser scanning

microscopy, Wu et al. (2015) showed that vaginal *L. crispatus* is also able to form a typical biofilm, with distinct developmental phases and architecture characteristics. However, phenotypic studies of *L. crispatus* strains by crystal violet assays revealed no or weak biofilm formation, as well as very low levels of autoaggregation (van der Veer et al., 2019), which shows a need for further studies on the possibility of formation of biofilm by *L. crispatus* and all the factors involved (Donnarumma et al., 2014; Wu et al., 2015; van der Veer et al., 2019).

Using adhesion competition assays in HeLa cell culture, Castro et al. (2013) observed the ability of *L. crispatus* EX533959VC06 to reduce the adhesion of *G. vaginalis* to host cells. The larger size of *L. crispatus* cells may be one of the factors that interfere in the adhesion of *G. vaginalis*. In addition, *L. crispatus* could inhibit adherence of BV-causing *G. vaginalis* through steric hindrance or by masking receptors. The *Lactobacillus* epithelium adhesin (LEA) of *L. crispatus* was characterized in the chicken-isolated strain ST1, which is known for its strong adherence to chicken epithelia and to buccal and vaginal cells of human origin (Edelman et al., 2002, 2003). The LEA protein displays specific binding to both crop epithelium and epithelial cells in the human vagina (Edelman et al., 2012). A comparative genomic study of Ojala et al. of 10 *L. crispatus* isolates found that the core genome of this species includes genes that may play a role in competitive exclusion of *G. vaginalis*. The study findings indicate that *L. crispatus* could interfere with fibronectin-binding and pilus components of *G. vaginalis*, promoting reduction in the ability of *G. vaginalis* to adhere to epithelial cells, which possibly helps in the prevention of BV (Ojala et al., 2014). A microbiome study that focused on bactericidal and bacteriostatic features of *L. crispatus* found that all of the eight analyzed strains from lactobacilli-dominated microbiomes and strains from microbiomes containing BV-associated bacteria encoded the LEA protein (Abdelmaksoud et al., 2016).

Antimicrobial activity

Vaginal lactobacilli play a key role in the inhibition of growth of other bacterial species through the production of antimicrobial substances, such as lactic acid, hydrogen peroxide, bacteriocins, bacteriocin-like substances and biosurfactants (Amabebe and Anumba, 2018). It is believed that genes found to be exclusive to the *Lactobacillus*-dominated microbiomes could play a role in the maintenance of vaginal health, while genes found to be exclusive to BV-associated bacteria-containing microbiomes could play a role in the development of BV (Abdelmaksoud et al., 2016).

Lactic acid production

Lactic acid at physiological concentrations acidifies vaginal secretions, enhances the protective activities of H_2O_2 and bacteriocins, and inhibits opportunistic infections (Cadieux et al., 2009; Thoma et al., 2011; Amabebe and Anumba, 2018). It has been postulated that proliferation of vaginal lactobacilli is supported by estrogen-driven glycogen production and that the core genome of *L. crispatus* does not contain the necessary genes to break down glycogen. Thus, it has even been suggested that *L. crispatus* relies on amylase secretion by the host or other microbes for glycogen breakdown (Amabebe and Anumba, 2018). However, Van der Veer et al. (2019) provided the first evidence suggesting that *L.*

crispatus human isolates can grow on extracellular glycogen and identified variation in a gene correlated with this activity. Anaerobic metabolism of glucose and other sugars results in the production of the isomers of lactic acid and energy production in the form of adenosine triphosphate. The vaginal epithelium produces only L-lactic acid, and in small amounts, while lactobacilli are the main source of both L- and D-lactic acid (Amabebe and Anumba, 2018).

Lactobacillus crispatus encodes two L-lactate dehydrogenase genes and one D-lactate dehydrogenase gene. Analyzing strain-to-strain differences in relation to total lactic acid production *in vitro*, no consistent or significant difference were observed between *L. crispatus* isolates from *Lactobacillus*-dominated vaginal microbiomes and isolates from microbiomes containing bacterial vaginosis-associated bacteria (Ojala et al., 2014; Abdelmaksoud et al., 2016; van der Veer et al., 2019). Compared to other species, *L. jensenii* is similar to *L. crispatus* in harboring two D-lactate dehydrogenase genes and one L-lactate dehydrogenase gene, whereas *L. iners* only harbors one D-lactate dehydrogenase gene. The genomic differences related to lactic acid and the transcriptional control of these genes may explain clinical observations in which *L. jensenii* and *L. crispatus* are associated with a healthy vaginal microbiome, in contrast to *L. iners*, which is frequently found in women with recurrent BV (Witkin et al., 2013; Ojala et al., 2014; Abdelmaksoud et al., 2016).

It has been suggested that the relative proportion of L- to D-lactic acid isomers in the vagina influences the role of *L. crispatus* and other lactobacilli such as *L. jensenii*, against BV (Amabebe and Anumba, 2018). An increase in the concentration of D-lactic acid relative to the L-lactic acid level correlated significantly to a decrease in extracellular metalloproteinase inducer (EMMPRIN) concentration (Witkin et al., 2013). EMMPRIN is the main inducer of matrix metalloproteinase (MMP-8), an enzyme that degrades the extracellular matrix (Iacono et al., 2007). EMMPRIN-induced MMP-8 has been strongly implicated in controlling endometrial breakdown and regeneration during the menstrual cycle (Braundmeier et al., 2006). In addition, EMMPRIN is an essential cofactor for protein monocarboxylate transporter 1 (MCT-1) (Wilson et al., 2005). MCT-1 and EMMPRIN-mediated mechanisms regulate only the intracellular concentration of the L-lactic acid isomer, through active transport into and out of epithelial cells, for maintenance of acidity compatible with cellular function (Iacono et al., 2007). Lower levels of EMMPRIN-induced MMP-8 may be related to increased integrity of the endocervical barrier that prevents vaginal microorganisms from ascending to the upper genital tract (Rahkonen et al., 2009). Thus, D-lactic acid may prevent BV-related complications and diseases by modulating the L-lactic acid-induced production of EMMPRIN from vaginal epithelial cells and inhibiting the production of MMP-8 (Witkin et al., 2013).

While D-lactic acid levels have been found to be strongly associated with the predominance of *L. crispatus* in the vagina, reduced levels of D-lactic acid have been associated with bacterial communities dominated by *L. iners* and *G. vaginalis* (Witkin et al., 2013). Since *L. crispatus* strains harbor two L-lactate dehydrogenase genes and one D-lactate dehydrogenase gene, further studies of transcriptional analyses should be conducted to help explain the association of *L. crispatus* with a higher level of D-lactic acid compared to L-lactic acid in the vaginal environment, (Witkin et al., 2013; Ojala et al., 2014; Abdelmaksoud et al., 2016; Amabebe and Anumba, 2018).

Hydrogen peroxide production

There is a controversy about the protective role ascribed to hydrogen peroxide (H_2O_2) producing *Lactobacillus* species. The protective role attributed to H_2O_2 is related to observations of epidemiological studies examining the presence of H_2O_2-producing versus non-producing vaginal lactobacilli was compared. The H_2O_2-producing lactobacilli were associated with a decreased risk for BV, sexually transmitted infections, and adverse birth outcomes. However, the scientific literature does not support a clear antimicrobial role for H_2O_2 produced by vaginal lactobacilli (Tachedjian et al., 2018).

A comparative genomic study by Ojala et al. (2014) discovered hydrogen peroxide producing enzymes (EC:1.2.3.3 and EC:1.1.3.15) in all of the 10 strains of *L. crispatus* that they analyzed (Ojala et al., 2014), which confirms experimental observations that hydrogen peroxide generation is common in vaginal *L. crispatus* (Antonio et al., 1999). However, there is weak correlation between stability or protection conferred by a *Lactobacillus* strain and the levels of hydrogen peroxide that it produces (Abdelmaksoud et al., 2016). Although strains of *L. vaginalis* and *L. jensenii* are the largest producers of hydrogen peroxide on vaginal microbiota, *L. crispatus* appears to be the most stable and protective species (Tamrakar et al., 2007); even hydrogen peroxide-non-producing strains of *L. crispatus* appear to play a protective role (Verstraelen et al., 2009). Unlike the other *Lactobacillus* species, *L. iners* is unable to produce D-lactic acid and H_2O_2, which may help to explain their frequent presence in women with recurrent BV (Petrova et al., 2017). Thus, the production of hydrogen peroxide does not seem to be a determining factor for the protective role of *L. crispatus*, but rather an enhancer when combined with other substances such as D-lactic acid.

Bacteriocins

All of 18 strains characterized in genomic studies by Ojala et al. (2014) and Abdelmaksoud et al. (2016) presented sets of putative bacteriocin gene clusters. In each isolate, at least two regions encoding bacteriolysins (class III bacteriocins) similar to enterolysin A (Nilsen et al., 2003) and helveticin J (Joerger and Klaenhammer, 1990) were revealed, except for one strain that lacked a gene similar to enterolysin A. Regions involved in the production of class II bacteriocins were also identified, such as a pediocin-like bacteriocin, penocin A, which inhibits the growth of pathogenic *Listeria* and *Clostridium* species (Diep et al., 2006), and bacteriocin LS2, which inhibits the growth of isolates belonging to the genera *Listeria*, *Shigella*, and *Yersinia* (Busarcevic and Dalgalarrondo, 2012). Besides these bacteriocins, thermophilin A, durancin Q, coagulin A and staphylococcin C55β were also found (Abdelmaksoud et al., 2016). The large number of putative bacteriocin gene clusters suggests the importance of bacteriocins for the antimicrobial potential of *L. crispatus*. However, no correlation was found between the number or type of bacteriocins in *Lactobacillus*-dominated vaginal microbiomes or isolates from dysbiotic vaginal microbiomes (Ojala et al., 2014; Abdelmaksoud et al., 2016; van der Veer et al., 2019).

Lactobacillus crispatus in the vaginal probiotic approach

The probiotic potential of the *L. crispatus* strains has been extensively studied. However, few clinical studies have focused on the analysis of the probiotic role of a single strain for prevention or treatment of BV (Table 2).

L. crispatus GAI 98332, *L. crispatus* CTV-05 (LACTIN-V) and *L. crispatus* IP 174178 (Physioflor®) were subjected to single strain clinical trials aiming at prevention and treatment against BV or UTI, which has potential against BV as well. *L. crispatus* GAI 98332 was selected among three strains of vaginal origin as the most suitable strain for a pilot study due to a higher level of hydrogen peroxide production. In addition, this strain showed great stability when frozen (Uehara et al., 2006). So, vaginal suppositories containing 1×10^8 CFU *L. crispatus* GAI 98332 were tested the safety and effectiveness in women experiencing recurrent UTI. A significant reduction in the number of recurrences was noted, without any adverse complication (Uehara et al., 2006). Ten years later, a report was published of an on-going prospective phase 2 clinical trial performed to evaluate the preventive effectiveness of *Lactobacillus* vaginal suppositories for prevention of recurrent cystitis (Wada et al., 2016). Female outpatients with recurrent cystitis were instructed to insert a vaginal suppository containing 1×10^8 CFU of *L. crispatus* GAI 98332 every two days or three times a week for one year before going to bed (Wada et al., 2016), as described in Uehara et al. (2006).

L. crispatus CTV-05 is the strain with the largest number of studies available for prevention and treatment against BV or UTI, with a biotechnological product available, the LACTIN-V developed by Osel, Inc. (Santa Clara, CA). Previously, *L. crispatus* strain CTV-05, a vaginally derived H_2O_2-producing strain, showed high mean adherence to vaginal epithelial cells *in vitro* (Kwok et al., 2006) and a high success rate in vaginal colonization when given as a vaginal suppository (Antonio and Hillier, 2003), which qualified this strain as a promising probiotic candidate. Furthermore, it was reported that this strain has a specific DNA fingerprint that allows it to be distinguished from endogenous vaginal lactobacilli, including other *L. crispatus* strains, and the success of colonization can be measured (Antonio and Hillier, 2003).

Therefore, LACTIN-V was developed as a vaginal suppository at a dose of 5×10^8 CFU administered in a gelatin capsule. A phase 1 trial was conducted in premenopausal women with a history of recurrent urinary tract infection to further evaluate the safety of this new formulation and its effect on the vaginal ecosystem. Only minimal side effects were observed; however, *L. crispatus* CTV-05 colonization was lower than expected and mild inflammation of the urinary tract was noted in some women (Czaja et al., 2007).

In another study, two different doses (10^6 and 10^8 CFU) in gelatin capsules containing *L. crispatus* CTV-05 in a desiccated state were evaluated for safety and vaginal colonization ability in young women. Overall, it was observed that the factors that predict failure in colonization by probiotic lactobacilli include vaginal intercourse, exposure to semen, and the presence of lactobacilli of the same species. Sexual intercourse with the use of condoms affected colonization, but unprotected sex affected it even more, possibly due to the presence of seminal fluid. A higher success rate in vaginal colonization was found in females not previously colonized by *L. crispatus.* Thus, the competition with endogenous *L. crispatus* appears to preclude the successful colonization by an exogenous *L. crispatus* strain (Antonio et al., 2009).

Table 2. Clinical studies of single-strain *Lactobacillus crispatus* in a probiotic approach. UTI = urinary tract infection**.**

Intervention	Health Condition	Results	Reference
Pilot study: Vaginal suppository containing 10^8 CFU of *L. crispatus* GAI 98332, every two days, for one year	Female patients aged 37-80 years who had experienced more than two episodes of UTI in the preceding 12 months, and were suffering from recurrent UTI for at least two years	A significant reduction in the number of recurrences was noted, without adverse complications	(Uehara et al., 2006)
Intravaginal capsule containing 10^8 CFU of *L. crispatus*, twice daily for three days, monthly for three months	Womens aged 18–45 years with BV and treated with a single oral dose of metronidazole (2.0 g) at the time of enrollment	Adverse effects were rare. Report of satisfaction with the capsule, belief that it contained healthy bacteria, and belief that its use improved vaginal health were directly related to clinical cure and to improved Nugent score.	(Marrazzo et al., 2006)
Phase 1 clinical trial: Vaginal suppository containing 5×10^8 CFU of *L. crispatus* CTV-05 (LACTIN-V) in a gelatin capsule, once daily for five days	Premenopausal womens aged 18–35 years with a history of three or more uncomplicated UTIs diagnosed in the past year, or two uncomplicated UTIs diagnosed in the past six months	*L. crispatus* CTV-05 was well tolerated and minimal side effects was observed, but the colonization was lower than expected and mild inflammation of the urinary tract was noted in some women	(Czaja et al., 2007)
Vaginal gelatin capsules containing two potencies (10^6 or 10^8 CFU) of *L. crispatus* CTV-05 in a desiccated state, twice daily for 3 days	Sexually active females aged 14-21 years and free of genital infections	No statistically significant difference was found between the two potency groups with respect to vaginal colonization by *L.crispatus* CTV-05. The factors that predict colonization failure include exposure to semen, vaginal intercourse, and the presence of lactobacilli endogenous of the same species	(Antonio et al., 2009)
Phase 1 clinical trial (ClinicalTrials.gov NCT00537576): Vaginal applicators prefilled with three doses (5×10^8, 1×10^9 or 2×10^9 CFU/dose) of *L. crispatus* CTV-05 (LACTIN V), once daily for five consecutive days	Sexually experienced healthy females aged 18-40 years, sexually-abstinent (sexually abstinent 72 hours prior to enrollment and until the last clinical visit)	All three dose levels of LACTIN-V appeared to be safe and acceptable in pre-menopausal healthy women	(Hemmerling et al., 2009)
Phase 2a clinical trial: (ClinicalTrials.gov NCT00635622) Vaginal applicator prefilled with 2×10^9 CFU/dose of *L. crispatus* CTV-05 (LACTIN V), once daily for five days followed by once weekly for two weeks	Women aged 18–50 years, premenopausal, diagnosed with BV and treated with 0.75% topical metronidazole (MetroGel) for five consecutive days before enrollment	LACTIN-V colonized well, and was safe and acceptable	(Hemmerling et al., 2010) In continuation of the Hemmerling et al. phase 1 clinical trial (Hemmerling et al., 2009)
Phase 2 clinical trial (ClinicalTrials.gov NCT00635622): Vaginal applicator prefilled with 2×10^9 CFU/dose of *L. crispatus* CTV-05 (LACTIN V), once daily for five days followed by a weekly application over two weeks	Women aged 18–44 years, premenopausal, diagnosed with BV and treated with 0.75% topical metronidazole (MetroGel) for five consecutive days before enrollment	Vaginal concentration of certain BV-associated bacteria, vaginal intercourse during treatment, and the presence of endogenous *L. crispatus* at enrollment predict colonization with probiotic lactobacilli	(Ngugi et al., 2011) In continuation of the Hemmerling et al. phase 1 and phase 2a clinical trials (Hemmerling et al., 2009, 2010)
Phase 2 clinical trial (ClinicalTrials.gov NCT00305227): Vaginal suppository (gelatin capsules with no applicator) containing 10^8 CFU/dose of *L. crispatus* CTV-05 (LACTIN V), daily for five days and then once weekly for 10 weeks	Women aged 18–40 years with history of at least one prior symptomatic UTI treated within the past 12 months prior to the current UTI (the participants were treated for acute UTI at visit 1)	The administration of LACTIN-V, after treatment for cystitis, provided an apparent treatment advantage over natural recovery of the vaginal microbiota and was associated with a reduction in recurrent UTI	(Stapleton et al., 2011) In continuation of the Czaja et al. phase 1 clinical trial (Czaja et al., 2007)
Phase 2 clinical trial: Vaginal suppository containing 10^8 CFU of *L. crispatus* GAI 98332, every two days or three times a week for one year	Female outpatients, less than 80 years old, with two or more episodes of uncomplicated/complicated cystitis in the past year (the UTI is treated and cured at entry)	An on-going prospective phase	(Wada et al., 2016) In continuation of the Uehara et al. pilot study (Uehara et al., 2006)
Phase 3 clinical trial: *L.crispatus* IP 174178 (Physioflor®), administered at a dose 10^9 CFU per gram in vaginal capsules once a day, for 14 days over the first two menstrual cycles and another 14 days for the following two menstrual cycles	Women with at least two documented episodes of BV in the previous year who had been clinically cured after oral metronidazole treatment (1 g/day x 7 days)	In women with recurrent BV after antibiotics, Physioflor® could reduce the rate of recurrence and increase the time to recurrence	(Bohbot et al., 2018)

To improve vaginal colonization by LACTIN-V, Osel Inc. increased the product dose and designed a novel applicator to facilitate delivery of the powder formulation directly into the vagina. A phase 1 dose-ranging safety trial (ClinicalTrials.gov

NCT00537576) tested the safety, tolerability and acceptability of three different doses of this new LACTIN-V formulation using the new vaginal delivery device in healthy volunteers. All three dose levels of LACTIN-V (5×10^8, 1×10^9 and 2×10^9 CFU/dose) administered by vaginal applicator appeared to be safe and acceptable in healthy volunteers (Hemmerling et al., 2009).

Posteriorly, a phase 2a clinical trial (ClinicalTrials.gov NCT00635622) assessed colonization efficiency, safety, tolerability, and acceptability of LACTIN-V administered at a dose of 2 x 10^9 CFU by a vaginal applicator in women diagnosed with BV and treated with 0.75% topical metronidazole (MetroGel). LACTIN-V promoted satisfactory colonization of the vaginal environment with safety, tolerability and acceptability. Due to the small sample size, the study was not effective to evaluate recurrence of BV (Hemmerling et al., 2010). Although the influence of semen exposure on colonization was not an initial aim of the study, a negative effect of unprotected intercourse on lactobacilli colonization was also found, which supports the conclusions made by Antonio et al. (2009) and Hemmerling et al. (2010). In the same clinical trial (ClinicalTrials.gov NCT00635622), a phase 2 study investigated whether the vaginal concentration of certain BV-associated bacteria could affect colonization with exogenous *L. crispatus* CTV-05. LACTIN-V at 2×10^9 CFU/dose was administered vaginally via a pre-filled applicator in women with BV previously treated with MetroGel®. *L. crispatus* CTV-05 colonization status inversely correlated with vaginal concentrations of BV-associated bacteria DNA, especially those known to create a biofilm (Ngugi et al., 2011).

As previously mentioned in the literature, vaginal intercourse and endogenous *L. crispatus* seems to negatively influence the success of colonization by *L. crispatus* CTV-05. It has been suggested that the high pH of seminal fluid or one of its components may affect the adherence of CTV-05 to vaginal epithelial cells and/or its survival in the vaginal environment (Antonio et al., 2009; Hemmerling et al., 2010; Ngugi et al., 2011). The presence of endogenous *L. crispatus* at enrollment was found to be significantly associated with a reduced probability of colonization with *L. crispatus* CTV-05, which supports the hypothesis of competition between endogenous and exogenous *L. crispatus* (Antonio et al., 2009; Ngugi et al., 2011).

Posteriorly, considering the clinical trial published by Czaja et al. (2007), a phase 2 trial (ClinicalTrials.gov NCT00305227) was conducted among women with recurrent urinary tract infection. LACTIN-V was administered as an intravaginal suppository, at a dose of 10^8 CFU/mL in a gelatin capsule, for prevention of recurrent urinary tract infection in premenopausal women. The administration of LACTIN-V after treatment for cystitis was associated with a reduction in recurrent urinary tract infection. Using quantitative qPCR of 16S ribosomal ribonucleic acid, a robust and prolonged colonization with *L. crispatus* CTV-05 was found, which resulted in a trend of reduction in the incidence of recurrent urinary tract infection by ~50%. Furthermore, the protective effects appeared to be proportional to increased colonization, since the protective effects were even greater in those women who achieved the most robust colonization with *L. crispatus* CTV-05. Therefore, after an episode of recurrent urinary tract infection, the administration of LACTIN-V provided an apparent treatment advantage over natural recovery of the vaginal microbiota (Stapleton et al., 2011).

L. crispatus IP 174178 was also analyzed to prevent BV. Women with recurrent BV after use of antibiotics were treated with *L. crispatus* IP 174178 (Physioflor®), administered

at a dose 10^9 CFU per gram in vaginal capsules. Despite the limitations of the study, Physioflor® slightly reduced the recurrence rate and increased the time to recurrence. The authors emphasized that the use of Physioflor may be a factor that assists in the prevention of BV, and that in order to sustain a long-term benefit, the known risk factors of BV should be considered (Bohbot et al., 2018).

CONSIDERATIONS AND PERSPECTIVES

The increasing number of studies correlating BV with other diseases and complications highlights the importance of further research on this syndrome. An ineffective initial response to antimicrobial therapy and high rates of BV recurrence in responsive patients can be controlled with a *L. crispatus* probiotic approach. The competitive exclusion and antimicrobial potential of *L. crispatus* is mediated by strain-specific genetic and transcriptional factors. Some *L. crispatus* probiotic strains are already commercially used, but other factors related to BV recurrence should also be considered concomitantly with the use of probiotics. *L. crispatus* CTV-05 is the most widely studied probiotic strain for treatment of BV. Despite the promising results of the use of LACTIN-V (*L. crispatus* CTV-05) as a probiotic, the inability of constant recolonization of the vaginal environment due to the presence of domestic *L. crispatus* signals a need for further genomic, transcriptome, proteomic and interatomic studies to help understand the host-probiotic and probiotic-probiotic interactions. Next-generation sequencing technology has increased knowledge about the vaginal microbiota and genomics of *L. crispatus*. More functional studies should be made to understand the performance of these probiotic bacteria in vaginal environment and to contribute to the screening of strains with the greatest potential for probiotic effects. Posteriorly, the best probiotic strains can still be further improved by genetic engineering techniques, acquiring new attributes to enable them to enhance their beneficial characteristics.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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