

## INVITED REVIEW

# Probiotic lactobacilli in formulas and hygiene products for the health of the urogenital tract

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## Abstract

Lactobacilli are the predominant microorganisms of the healthy human vagina. A novel alternative for the prevention and treatment of female urogenital tract infections (UGTI) is the inclusion of these microorganisms as active pharmaceutical ingredients in probiotic formulas, and more recently in female hygienic products. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” A list of requirements must be considered during the development of probiotic product/formula for the female urogenital tract (UGT). This review aims to resume the requirements, probiotic characteristics, and clinical trial applied to determine the effect of probiotic and potentially probiotic strains on different woman’s physiological and pathological conditions, and in preterm birth prevention. A revision of female hygienic products available in the world market is included, together with novel studies applying nanotechnology for *Lactobacillus* incorporation in hygienic products. Further studies and well-designed clinical trials are urgently required to complement the current knowledge and applications of probiotics in the female UGT. The use of probiotic formulas and products will improve and restore the ecological equilibrium of the UGT microbiome to prevent and treat UGTI in women under different conditions.

## KEYWORDS

clinical trials, female hygienic products, female urogenital tract, live biotherapeutic products, probiotics, urogenital tract infections

## 1 | HEALTHY VAGINAL MICROBIOTA— LACTOBACILLUS PREDOMINANCE: FUNCTIONS

The human body and their mucosa are lately considered as highly active ecosystems, where microorganisms play a very diverse list

of functions and contribute to host nutrition, overall development, defenses, and immune system, response to pathogens, and mucosal cell differentiation and proliferation. The related research and the knowledge of these active communities and their gene contents have been referred collectively as the human microbiome (HM), supported mainly by an NIH-funded project consortium.<sup>1</sup> The Human

**Abbreviations:** AV, aerobic vaginitis; BV, bacterial vaginosis; DNA, deoxyribonucleic acid; GBS, Group B *Streptococcus*; GDM, gestational diabetes mellitus; GRAS, generally regarded as safe; HIV, human immunodeficiency virus; HM, human microbiome; HPV, human papilloma virus; HR-HPV, high risk human papilloma virus; HSV-2, Herpes simplex virus type 2; IM, intestinal microbiota; LBP, live biotherapeutic products; LN, Lacto Naturel; NIH, national institutes of health; PPROM, preterm premature rupture of membranes; QPS, qualified presumption of safety; R-VVC, recurrent-VVC; SR, slow-release; TV, trichomoniasis; UD, undetermined; UGT, urogenital tract; UGTI, urogenital tract infections; UTI, urinary tract infection; VM, vaginal microbiota; VVC, vulvovaginal candidiasis.

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Microbiome Project and the European MetaHIT consortium initiated almost two decades ago, aimed at detailed characterization of the structure and the composition of the microbiota from various body areas.<sup>1</sup> It is interesting to remark that microbial numbers within an individual are estimated higher than the human cell number by an order of magnitude. In such a way that the HM is a complex system of many microbial communities, deeply described in terms of composition, diversity, and dynamics, known thanks to the use and update of massively parallel sequencing and other high throughput approaches available, indicating the long list of eukaryotes, archaea, bacteria, and viruses detected. These molecular techniques include Sanger sequencing of 16S rRNA of bacterial colonies, terminal restriction fragment length polymorphism of 16S rRNA, qPCR, and next-generation sequencing that have modified the concept of the microbiome versatilities and more specifically *Lactobacillus* identification.<sup>2-13</sup>

Then, the majority of the indigenous microbiota exists in a mutually beneficial relationship with the host, while few are opportunistic pathogens.<sup>14</sup> An important body site providing a habitat for the development of structured microbial communities is the vaginal tract, which is broadly colonized by microorganisms known as the vaginal microbiota (VM). Unique conditions of the vagina are characterized by a few microbial species, being the vaginal microbiome a specific compartment of the HM.<sup>15</sup> The predominance of lactobacilli was described deeply in the vaginal tract in the NIH Project, and this concept agrees with the first proclaimed by Doderlein in the early 1900.<sup>16</sup> The most frequently isolated species are *Lactobacillus crispatus*, *L. gasseri*, *L. jensenii*, and *L. iners*. Their relative dominance was studied by Ravel et al.<sup>8</sup> in different races and ethnic groups. They evidenced the prevalence of *L. crispatus* (group I), *L. gasseri* (group II), *L. iners* (III), and *L. jensenii* (V) in 26.2%, 6.3%, 34.1%, and 5.3% of the women sampled, respectively. A large heterogeneous group (IV) was presented in 27% of the women with a higher proportion of strictly anaerobic bacteria (*Prevotella*, *Dialister*, *Atopobium*, *Gardnerella*, *Megasphaera*, *Peptoniphilus*, *Sneathia*, *Eggerthella*, *Aerococcus*, *Finegoldia*, and *Mobiluncus*). These results indicate that a potential key ecological function, the production of lactic acid, seems to be conserved in all communities, and support at the same time the application of lactic acid producer and immunomodulatory-probiotic bacteria, and are concordant with  $\alpha$  diversity studies published later.<sup>17-19</sup> Ma and Li<sup>20</sup> have published recently a review on the association between vaginal community state types and species specificity index reclassifying in five groups.

The second phase of the NIH-Human Microbiome Project was the Integrative Human Microbiome Project<sup>21</sup> designed to explore host-microbiome interplay, including immunity, metabolism, and dynamic molecular activity to gain a more holistic view of host-microbe interactions over time, in a way to address the relationships between host and microbiome mechanistically. The enormous importance of vaginal microbiome supported its inclusion as one of the systems proposed to be studied in this second phase: pregnancy and preterm birth, to go further in the dynamics of human health and disease-related with

known microbiome interactions. In such a way that the concept of *reproductive microbiome* has been conceived, joining the microbiome of the vaginal tract, placenta and milk/mammary gland, and the direct relationship with fetal development, birth, and newborn features.<sup>22</sup> In addition, the VM of the mother plays an essential role in the initial colonization of newborn babies and therefore the development of a healthy gastrointestinal and skin microbiota. The maternal microbiota exerts an indirect effect on the fetus via maternal factors, such as maternal immune responses or microbial metabolites that cross the placenta,<sup>23,24</sup> or other indirect factors mediating epigenetic programming in the fetus (diet, stress, or neuroendocrine exposure). Kaminska and Gajecka<sup>25</sup> discuss the influence of human VM, not only bacteria but also viruses and fungi that constitute important components of the reproductive tract microbiome. The impact of the maternal microbiome on fetal development, and the establishment of neonatal microbiomes, including the placenta microbiome, and the hematogenous source of intrauterine infection on the health status of women were analyzed. On the other side, some evidence indicates that infertile patients harbor a different reproductive tract microbiome compared with healthy and fertile women.<sup>26</sup>

Fertilization occurs in the uterus, an immune-protected organ, considered a sterile site maintained by the cervical plug for centuries. But the microbial communities in the endometrial cavity and its implications in reproductive health and disease, particularly chronic endometritis was published.<sup>27</sup> Even though bacterial DNA in blood in placenta was demonstrated, mechanisms and functions of transplacental trafficking of free nucleic acids are still in discussion. Then, microbes interact with the host cells along the female reproductive tract, generating the physical, chemical, and biological environment that embryo will encounter during the peri-implementation period and throughout pregnancy. Later, birth represents the first major exposure to a complex microbiota, because the birth canal is always adjacent to the rectum, providing an efficient mechanism for intergenerational transmission of both vaginal and gut microbes.<sup>28</sup> The baby swallows these microorganisms, supported by DNA and live bacteria in the meconium.<sup>29</sup> The maternal gut microbes immediately start to colonize the newborn's own gut, engaging in a kind of conversation with developing immune cells. In this way, the very early microbiome prepares the immune system for healthy functioning later in life. When a baby is born by cesarean section, the gut is seeded with different microbes not those from the mother's gut and vagina, but from her skin and breast milk, the nurse's hands, and other sources. These early differences might have implications that last a lifetime and provides differential colonization and diversity of microorganisms in the intestinal microbiome in C-sections or vaginal born babies later in their lives.<sup>30,31</sup>

On the other hand, the urinary tract, previously considered a sterile body niche, has emerged as the host of an array of bacteria in healthy individuals, revolutionizing the urology research field.<sup>32</sup> Specific bacterial communities have found in the healthy urinary tract, which can change in a wide variety of urologic disorders. Then, it is also of main importance to resolve the modulation of the microbiome to improve urinary tract health.

## 2 | FACTORS AFFECTING THE VM EQUILIBRIUM

The equilibrated/healthy ecological systems of the reproductive microbiota can be affected by a long list of intrinsic or extrinsic factors. *Intrinsic factors or host factors* include race, physiologic (hormonal changes, menstruation, pregnancy), immune system imbalances, maturation, and the relationship with genetic susceptibility, cancer, and the phages isolated in the tract.

The VM of reproductive-age women was separated according to the *ethnic groups* (White, Black, Hispanic, and Asian) in five clusters, showing that the proportions of each community group varied among them.<sup>8</sup>

Women's life is characterized by *continuous physiological changes*, from their birth through the reproductive age to menopause, and during all these phases the vaginal epithelium radically changes, and then the VM.<sup>33</sup> Monthly ovulation, with high estrogenic levels, lead to vaginal tract, particularly acid, optimum for the lactobacilli growth, producing lactic acid.<sup>33-35</sup> During the menstrual period there is an increment of pH in the area, and high availability of nutrients derived from menstrual bleeding for microbial growth. This period usually causes disturbance and discomfort, with lower number of lactobacilli, consequent undesirable microorganisms, increased infection rates, and recurrences.<sup>8,36,37</sup> In menopause, the estrogens are no longer present and glycogen level decreases, leading to a decrease in lactobacilli.<sup>38</sup>

The human *immune system* restricts microbiota to their natural niches.<sup>28</sup> There is growing evidence that the innate immune system-antimicrobial peptides and repertoire of pattern recognition receptors, evolved in response to the need for controlling the epithelium-colonizing microbiota.<sup>39</sup> The human vagina consists of multiple levels of protection in innate and adaptive immunity compartmentalize into various components.<sup>40,41</sup> On this subject, genetic variations, such as single nucleotide polymorphisms, in different genes coding components of immune system have been shown to modulate individual's susceptibility to acquire urogenital tract infections (UGTI).<sup>42</sup>

*Bacteriophages* are abundant members of the urogenital tract (UGT), most often persisting through the lysogenic life cycle as prophages integrated within the genomes of their bacterial hosts. Numerous prophages in vaginal lactobacilli were related as one of the factors affecting their depletion in vagina.<sup>43,44</sup>

### 2.1 | Extrinsic factors

A long list and variety of factors affect the microbiota equilibrium, as the addition of vitamins and folic acid to diet,<sup>45-47</sup> oral contraceptives,<sup>48</sup> sexual behaviors,<sup>49</sup> hygiene habits,<sup>50</sup> stress situations,<sup>51</sup> sexually transmitted diseases,<sup>52</sup> antibiotics,<sup>53</sup> or immunosuppressor therapies,<sup>54</sup> between others.

## 3 | UGTI AND FREQUENTLY APPLIED THERAPY

Female vaginal ecosystem thought to have been shaped over the years by co-evolutionary processes occurring between the particular microbial partners and the human host. Residing at the port of entry of pathogens, the vaginal *Lactobacillus* species can create a barrier against pathogen invasion since the main products of their metabolism secreted in the cervicovaginal fluid can play an important role in inhibiting invaders. Therefore, a *Lactobacillus*-dominated microbiota appears to be a good biomarker for a healthy vaginal ecosystem.<sup>55</sup> Disruptions in vaginal association with the microbiomes lead to the change in the vaginal environment, which enhanced the risk of acquiring diseases. The disturbed population promotes a loss of beneficial bacteria due to the list of exogenous/endogenous factors listed above. Thus, vaginal tract can be infected by diverse pathogens resulting in diseases such as bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), trichomoniasis (TV), urinary tract infections (UTI), aerobic vaginitis (AV), and sexually transmitted diseases as those caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*.<sup>14</sup> The main viral sexually transmitted diseases are human papilloma virus (HPV), human immunodeficiency virus, and herpes simplex virus type 2 (HSV-2). Each pathogen has its unique infection kinetics, pathology, and host evasion mechanisms. In a similar way, the genital mucosal immunity reacts differently toward each of them.

The urinary tract has emerged as the host of an array of bacteria in healthy individuals. There is a microbiome associated with the healthy urinary tract that can change in certain urological disorders.<sup>32,56</sup> The vagina is a key anatomical site in the pathogenesis of UTI in women, serving as a potential reservoir for infecting bacteria, and also the organ where the ascendant colonization from rectum begins.<sup>57</sup> UTIs in females usually start as vaginal infections and ascend to the urethra and bladder.<sup>58</sup> Women are affected with an estimated UTI lifetime risk of 60%, and 50%-fold predominance compared with adult men<sup>59</sup> increasing with age.<sup>60</sup> Single episodes are very common, being *Escherichia coli* the most common cause of UTI<sup>57,61</sup> or different Gram-positive bacteria.<sup>62</sup> Recurrent UTI occurring in up to one-third of women after the first episode,<sup>63</sup> showed to be related to a decreased vaginal lactobacilli.<sup>64</sup> Rates of UTI begin to rise at the climateric, and recurrences are one of the features of menopause.<sup>65</sup> Vaginal tract is then a site at which probiotic interventions may decrease the risk of UTI.<sup>57,58,66</sup>

A wide diversity of antimicrobial compounds is applied to treat UGTI by vaginal or oral administration, including antibacterial, antifungal, antiparasitic, and antiviral agents. The antibiotic use decreases the vaginal *Lactobacillus* number, promotes resistance, and sometimes infection recurrences. In such a way that the resistance to antimicrobials can be transmitted either transversally or horizontally to other members of the microbiome, generating multi-resistant microorganisms. The "*resistome*" concept has emerged, representing the collection and variety of genes that confer antimicrobial

resistance (biocides, heavy metals, plasmids) in different microbiomes, spread over multiple areas,<sup>67</sup> analyzed lately by targeted metagenomics techniques.<sup>68</sup>

For this reason, and supported by different concerns related to public health and the prevention of infections, the urgent requirement of adequate alternatives emerged in the last two decades.

## 4 | PROBIOTIC AS PROPOSAL THERAPIES. STRAIN, AND HOST SPECIFICITY

One of the therapies applied for UGTI are probiotics. The definition was submitted to many discussions by government and scientific organizations during the last 30 years, and at the end is *live microorganisms that, when administered in adequate amounts, confer a physiological health benefit on the host*.<sup>69,70</sup> However, more recently, new definitions have complemented the probiotic area. The term “paraprobiotics” means dead or inactive cells of probiotics that have shown a significant effect on human health<sup>71-74</sup>; while the term “postbiotics” is used to describe healthful metabolites of probiotics also showing effect on the host.<sup>75-78</sup> Lately, Zendeboodi et al.<sup>79</sup> proposed three main classes of probiotic products, including “true probiotic” as viable and active probiotic cell, “pseudo-probiotic” as viable and inactive cells in vegetative or spore forms, and “ghost probiotic” as dead/nonviable cell, either intact or ruptured.

Probiotics have been widely studied and applied in the field of food and food supplements, with no particular negative effect.<sup>80-82</sup> In the last few years, the pharmaceutical industry showed a growing interest in new formulations containing beneficial microorganisms, in many cases specific to the host.<sup>83,84</sup> However, the increased interest in the clinical application of probiotics requires specific attention by their administration to non-healthy population. More recently, the Food and Drug Administration has defined a new “live biotherapeutic products” (LBP) category. Then, the documents and demonstration of quality, safety, and efficacy of new products, including LBP, are framed by the characteristics and risks of the aimed population, as well as those of the strains and product components, in such a way that the global benefit–risk ratio can be assessed regarding their intended use.<sup>85</sup>

Not all the *Lactobacillus* strains can be considered as probiotics because the beneficial effect must be evidenced in the specific host as stated. The strains included in different products/formulas must be submitted to a long list of protocols and trials to be named as probiotics. Also, a body of different criteria applied, reviewed by different authors, to be considered as probiotic strains.<sup>69,86</sup> The range of functional genomics to identify genes and gene products that govern the distinctive phenotypes and health associations of probiotic strains was reviewed, recognizing the strain specificity of probiotic effects.<sup>87</sup> This strain specificity and the disease specificity of probiotic efficacy, and the need to take these two factors: both specific strains and type

of disease, when recommending the appropriate probiotic effect for each patient is also recommended.<sup>88,89</sup> One of the first considerations is the *host specificity*, which means that the strains should be isolated from the same host where will be applied. Even though some strains are used historically as probiotic or included in foods, the host specificity indicates a higher possibility to be adapted to the host conditions. Referred to the *vaginal tract or mucosal specificity*, Yildirim et al.<sup>90</sup> have shown that all primates exhibited host-specific VM and that humans were distinct from other primates in both microbiome composition and diversity. Mendes-Soares et al.<sup>91</sup> demonstrated that genomes of vaginal species were significantly smaller and had significantly lower GC content than those of non-vaginal species.

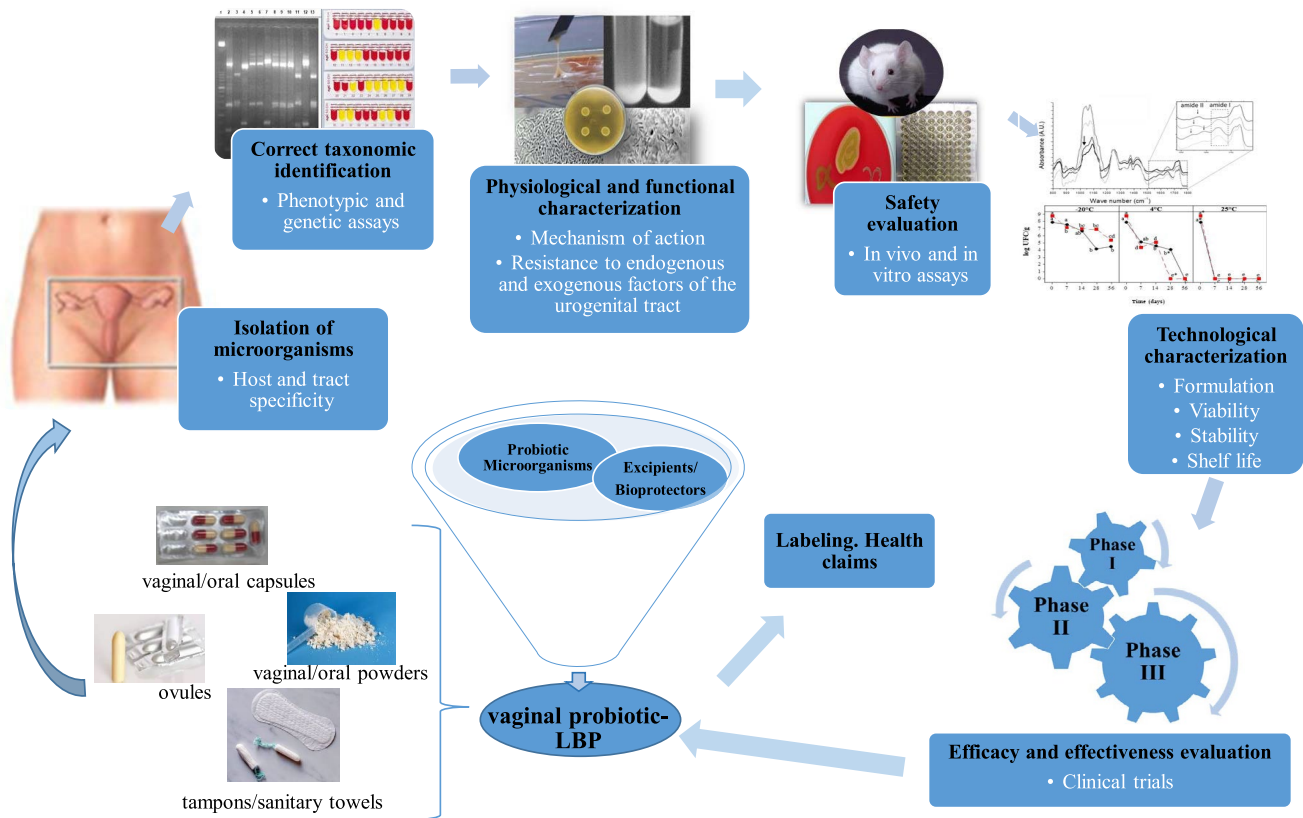
More specifically, Van Der Veer et al.<sup>92</sup> have found that *L. crispatus* from dysbiotic women have a more prevalent novel glycosyltransferase gene, while Pan et al.<sup>89</sup> evidenced strain-specific variations and phenotypic differences associated with the isolation source, either urogenital or gastrointestinal tracts.

## 5 | REQUIREMENTS FOR PROBIOTICS FORMULAS/PRODUCTS

In the design of probiotic formulas/products, different sets of criteria and protocols must be applied according to the requirements and exigencies of regulatory organisms, described in different revisions, added to the host and tract specificity described above.<sup>86,93,94</sup> They include: (a) place of isolation, (b) correct phenotypic and genetic identification of the strains, (c) functional characterization and mechanisms of action, (d) in vitro and in vivo safety evaluation, (e) technological characterization: production, formulation, and shelf life, (f) efficacy and effectiveness in clinical trials, and (g) label and health claims; and they are synthesized in Figure 1.

Most of the probiotic lactobacilli are considered as Generally Regarded as Safe and some specific species included in the Qualified Presumption of Safety (QPS) classification for inclusion in food,<sup>95</sup> but their effectiveness and safety characteristics are strain-specific and cannot be generalized. Then, no new strain should be assumed of sharing the same documented safety history with preexisting ones.<sup>96</sup> Different efficacy and safety assessment protocols were recommended by the experts for a putative probiotic candidate, before confirmation and acceptance for public consumption. In order to guarantee the probiotic safety, each strain must be assayed in some aspects: antibiotic-resistance patterns; assessment of certain metabolic activities; toxin production; determination of hemolytic activity; side-effects in experimental animal models; phase I trials; epidemiological surveillance of adverse incidents in consumers, between other.<sup>69</sup> Referred to the potential dissemination of the resistance mediated by genetic mechanisms, such as horizontal gene transfer where plasmids, transposons and integrons may be involved,<sup>97-101</sup> is essential to evaluate the susceptibility patterns of potentially probiotic bacteria.<sup>102</sup> Microbial resistance to clinically relevant antibiotics must be absent as part

## DESIGN AND ELABORATION OF VAGINAL PRODUCTS WITH BENEFICIAL PROBIOTIC BACTERIA



**FIGURE 1** Design and elaboration of vaginal products with beneficial probiotic bacteria (modified from reference [86]). LBP, live biotherapeutic products

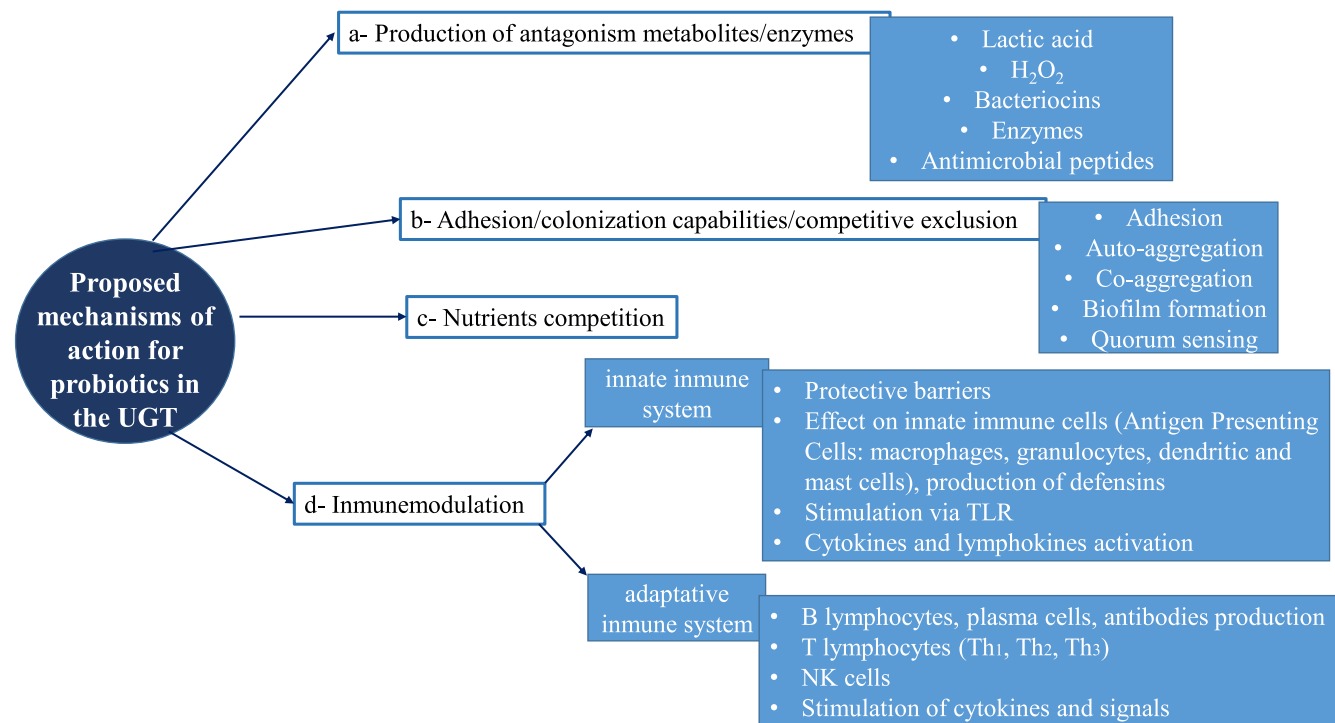
of the QPS assessment of bacteria deliberately introduced into the food chain.<sup>103</sup> Also it is important to screen the enzymes acting as potential virulence factors, as hydrolytic enzymes (hemolysin, lecithinase, gelatinase, etc.) able to produce damage to the host.<sup>104-107</sup> The temporal persistence and colonization or permanence capability of beneficial microorganisms, and their cellular and molecular effects on the integrity of host mucosa and immune system must also be assayed.<sup>108-110</sup>

The formulas for the UGT are designed for oral or local administration. The oral delivery is supported by the microbial ability to survive through gastrointestinal tract, and to ascend to vagina after their excretion from the rectum, in ascendant colonization.<sup>111-113</sup> Whereas vaginal application allows direct and targeted colonization of bacteria for the restoration of unbalanced urogenital microbiota.<sup>114-118</sup> The vaginal drug delivery systems include a wide variety of pharmaceutical forms such as liquid, semi-solid (gels, creams, ointments), and solid systems (tablets, vaginal suppositories, rings, films, tampons). The viable bacteria are incorporated frequently as powder, being lyophilization the gold standard technique for microbial preservation and stability during the storage for different time periods.<sup>119-122</sup> The selection of the appropriate dosage form depends on the physicochemical features of the delivered drug or formula, the target for them and women's acceptance.<sup>123</sup>

## 6 | EFFECTS OF PROBIOTIC OR POTENTIAL PROBIOTIC MICROORGANISMS ON THE FEMALE UGT

Pharmacological aspects of probiotics are more complex than those of conventional drugs, since their effect in the host cells-mucosa-organs and whole organism is very complex and difficult to evaluate, because they act by different mechanisms, either individually or in some cases synergistically. Several publications describe how vaginal lactobacilli can exert their function through different mechanisms of actions, which include: (a) production of antimicrobial substances (organic acids, hydrogen peroxide, bacteriocins, biosurfactants) or enzymes (e.g. arginine deaminase); (b) adhesion to epithelial cells, components of mucus or extracellular matrix-colonization-permanence; (c) autoaggregation and coaggregation; biofilm formation on vaginal mucosa; (d) competitive exclusion; (e) competition for nutrients; and (f) modulation of the immune system.<sup>86,94,124-132</sup> The proposed mechanisms of action of probiotics in the UGT are shown in Figure 2.

A very important subject to be considered in the development of probiotic formulas for the UGT is the effect of the bacteria as bioactive principle, whether for preventive or therapeutic purposes, which is supported by the reestablishment of the ecological microbial equilibrium of the tract. One of the main requirements



**FIGURE 2** Proposed mechanisms of action for probiotics in the UGT (adapted from different bibliographic references). TLR, toll-like receptor; UGT, urogenital tract

of probiotic products is the viable cell number, between  $10^7$  and  $10^9$  colony forming units per formulation dose.<sup>70,94,133,134</sup> Since the effectiveness of probiotic formulas depends on these number of viable cells, most of the pharmacokinetic studies have described the survival capability of probiotics in the target organ and its capability to maintain their numbers and colonize to generate the probiotic effects. The survival of microorganisms depends on their resistance to the conditions of the host tract and to the technological processes and their maintenance as marketed products. The fate of probiotics (i.e., their survival, or movement) in the gastrointestinal tract (or in experimental animal models), their effect in specific target organs, or stimulation of specific cell-populations, mainly in the gut were demonstrated. Scientific publications evidenced that oral probiotics increase intestinal antimicrobial activity and Paneth cells in order to strength the epithelial barrier against pathogens in mice, proposing a different mechanism by which probiotics protect the host mainly against infectious diseases. The whole bacteria can not enter the intestinal cells, while only the degradation products of bacteria are able to take contact with the immune cells.<sup>135,136</sup> A recent study have shown the probiotic properties of two vaginal lactobacilli (*L. fermentum* MG901 and *L. plantarum* MG989), the adhesion to HT-29 cell and the inhibition of *E. coli* and *Candida albicans* adherence to these cells. The probiotic bacteria persisted up to 6 days in the feces of mice after the oral administration. The authors suggested the vaginal strains could be used as oral and vaginal probiotic helping to in vivo clear VVC.<sup>137</sup>

Referred to oral probiotics for women, supported by the ascending colonization hypothesis that promotes the permanence

and colonization of beneficial bacteria in the vagina after excretion through the rectum,<sup>111-113</sup> the capability of microorganisms to colonize the vagina was studied. De Vrese et al.<sup>138</sup> showed that oral intake of four *Lactobacillus* strains improves the microbial pattern in vaginal dysbiosis. In a similar way, Vladareanu et al.<sup>139</sup> demonstrated that oral probiotic increased the vaginal colonization of lactobacilli via cross-contamination from the gastrointestinal tract to vagina, indicating an improvement of vaginal conditions, suggesting then the successfully prevention of VVC episodes.

On the other side, the effect, safety, and mechanisms of action of lactobacilli as probiotic products for local administration, such as vaginal capsules, suppositories, and tablets, were demonstrated in different publications. Verdenelli et al.<sup>140</sup> registered the normalization and maintenance of pH and Nugent score and the increase of lactobacilli total number after 7 days of probiotic suppositories treatment in women. The authors highlighted that local administration promotes a quick local action, driven by the activity of probiotic bacteria that adhere and colonize the vaginal epithelium. The colonization of vaginal epithelium was shown by molecular typing test, suggesting the restoration and maintenance of VM. Cohen et al.<sup>141</sup> published a randomized, double-blind, placebo-controlled, phase 2b trial to evaluate Lactin-V (*L. CTV-05*) after the treatment with vaginal metronidazole, resulting in a significantly lower incidence of BV recurrence.

The use of probiotics in the obstetrical and gynecological field has increased in the last years. Up to date, several clinical studies have evaluated the effects of probiotic or potential probiotic microorganisms, administered both orally and vaginally, on the prevention

and treatment of UGTI in women under different physiological conditions: fertile and non-pregnant, pregnant, and post-menopausal, as well as also in the prevention of preterm birth (Table 1). In the present review, most of the available clinical trials published between 2015 and 2020 were included, because previous trials were analyzed before.<sup>86,142</sup>

In non-pregnant, fertile women (Table 1, white panel), probiotic strains were assayed to: evaluate their safety, and to improve the VM of healthy women<sup>129,139,143,151</sup>; restore the abnormal VM<sup>129,140,159,165</sup>; prevent recurrent UGTI<sup>141,166</sup>; as adjunct treatment to BV,<sup>37,160,161,167</sup> AV<sup>160</sup> antimicrobial therapy, recurrent-VVC (R-VVC),<sup>152,162</sup> and TV in BV presence<sup>144</sup>; as BV and R-VVC subsequent treatment<sup>141,162,169</sup>; and HPV clearance.<sup>145,153</sup> Most of the publications have evaluated commercial, or potential probiotic formulas, including oral and vaginal capsules, vaginal tablets, suppositories or powders, and even yogurt, containing individual or combined *Lactobacillus* strains of urogenital, intestinal, or food origins, as shown in Table 1. *Lactobacillus* strains combined with bovine-lactoferrin were also assayed.<sup>37,151,152</sup> Independent of the administration route, the strain/s and doses evaluated, most of the trials reported positive effect on the UGT, such as the absence of adverse effects, VM improvement and restoration, permanence and colonization of probiotic microorganism, and higher cure rates and lower recurrences, mainly in BV, AV, R-VVC, and TV patients. However, Marcotte et al.<sup>161</sup> indicated that the probiotic adjunct therapy of BV did not improve cure rates or alleviate recurrence, probably due to treatment failures or to the limited power of the study. Referred to the use of probiotic for HPV clearance, Palma et al.<sup>145</sup> reported the higher HPV clearance in BV women with metronidazole therapy plus 6 months vaginal *Lactobacillus* implementation, than the one with only 3 months' use. Ou et al.<sup>153</sup> published that the application of probiotic strains did not influence genital high risk-HPV clearance, but decreased the rates of mildly abnormal and unsatisfactory cervical smears.

Probiotic application in pregnant women (Table 1, light gray panel) indicates that most of the clinical trials evaluated oral administration of commercial formulas containing urogenital *Lactobacillus* strains. A positive effect on the modulation of VM, increased beneficial microorganisms, and lower pathogen levels, as Group B *Streptococcus* (GBS) was reported.<sup>150,157,168</sup> However, oral probiotics taken from early pregnancy showed no effect on vaginal health during mid and end gestation,<sup>155,156</sup> or 3 weeks doses or until birth in 36-week-pregnancy were ineffective in impacting GBS vaginal colonization.<sup>158</sup> The addition of vaginal probiotics to standard antibiotic treatment on perinatal outcome in preterm premature rupture of membranes pregnancy prolonged the birth gestational age, the latency period length, improving newborn weight and health.<sup>163</sup> On the other hand, the oral application of probiotic in maternal health during pregnancy, postpartum, and in infant eczema and allergy prevention was evaluated.<sup>146-149</sup> *L. rhamnosus* HN001 during pregnancy reduced gestational diabetes mellitus (GDM) prevalence, particularly among older women and those with previous GDM,<sup>147</sup> and the depression and anxiety scores in women in the postpartum period.<sup>148</sup> However, *L. rhamnosus* HN001 did not reduce the infant eczema.<sup>149</sup>

In other way, Anoshina<sup>168</sup> showed that combined *Lactobacillus* strains to HSV-women decreased the complaints incidence of bloating, discomfort, constipation, mucus in stool, excessive vaginal discharge, itching, swelling and mucosa redness, reducing the placental insufficiency and preeclampsia as well as fetal distress incidence.

In postmenopausal women, the application of probiotic strains (Table 1, dark gray panel) in intimate care ointment demonstrated the successful colonization of probiotic strains to improve the healthy VM.<sup>164</sup> But, oral administration of probiotic+isoflavone was not as effective as the hormonal therapy to improve genitourinary menopause symptoms.<sup>170</sup>

In conclusion, several trials have demonstrated that single or combined probiotic or potential probiotic strains, either alone or supplemented to antimicrobial therapy, were effective for BV, AV, R-VVC, and TV prevention and treatment. However, there is insufficient clinical evidence on the efficacy of probiotics for vaginal health in pregnant, post-menopausal women, and for the prevention of preterm birth and its complications. Then, more studies with well-designed randomized controlled trials evaluating larger patient size, and different: length of interventions, dosage and strains of probiotics, and administration route are urgently required.

## 7 | HYGIENE PRODUCTS FOR THE PREVENTION OF UGTI

The probiotic administration in hygiene products, as tampons and pads, has emerged as a new possibility to carry beneficial strains and exert the claimed effect. The pharmacological aspects of the probiotic products for feminine hygiene including sanitary towels and tampons, classified in several countries as medical devices, are scarcely described. They can act in the VM restoration, and in some cases, could reduce the risk associated with toxic shock syndrome produced by *Staphylococcus aureus* strains, with higher incidence in women who use tampons.<sup>171</sup> The availability of everyday feminine hygiene products (e.g., tampons, sanitary napkins, panty liners) containing probiotic microorganisms from vaginal origin is limited in the market and available only in some regions of the world. Table 2 shows the feminine hygiene products containing vaginal probiotic lactobacilli assayed for pH balancing and protection during menstrual bleeding. Most of them were designed with Lacto Naturel, LN<sup>®</sup> formula, as tampons, sanitary pads, foams, and creams, with patented probiotic strains: *L. gasseri* LN40, *L. fermentum* LN99, *L. casei* ssp. *rhamnosus* LN113, *Pediococcus acidilactici* LN23. These strains were isolated from vagina and incorporated as freeze-dried cells. In tampons and towels, they are included in the inner part, and once hydrated, they diffuse to the surface in the vaginal cavity. Different trials of Ellen<sup>®</sup> probiotic tampons are included in the scientific summary, but the scientific-reviewed publications were not found.<sup>172</sup> This summary describes the clinical studies performed, indicating that the use of probiotic tampon can lead to vaginal colonization of LN<sup>®</sup> probiotic bacteria in asymptomatic women. These devices are aimed to prevent a disturbed VM condition, while the

TABLE 1 Effect of probiotic or potential probiotic microorganisms on the female urogenital tract

Microorganisms	Isolation site	Pharmaceutical form or product	Clinical target <sup>a</sup>	Type of study and participant type and number	Results	References
<i>Lactobacillus crispatus</i> CTV-05	Healthy human vagina	Vaginal powder (Lactin-V, Osel)	Subsequent to BV therapy	Multicenter randomized double-blind placebo-controlled phase 2b trial. 228 BV women	Probiotic (11 weeks) after metronidazole produced lower incidence BV recurrence	141
<i>L. plantarum</i> P17630	Healthy human vagina	Oral capsule (Proge Farm S.r.l.)	VM-improvement	Randomized double-blind-placebo-controlled study. 93 R-VVC history women	Probiotic [3 treatment cycles (15 days/cycle) separated by 15-day wash-out intervals] improved <i>Lactobacillus</i> colonization and clinical sign (redness/swelling/discharge)	139
<i>L. rhamnosus</i> Lcr35 <sup>®</sup>	Human feces	Vaginal capsule or slow-release (SR) vaginal tablet (Gynophilus <sup>®</sup> )	VM-improvement	Comparative phase I randomized open-label pilot clinical trial. 33 healthy women	Capsules (daily) or SR-tablets (every 3, 4, or 5 days for 21 days) did not produce adverse effects, favored <i>Lactobacillus</i> spp. growth, reduced non- <i>Lactobacillus</i> spp. colonization	143
<i>L. rhamnosus</i> BMX 54	UD	Vaginal tablet (NORMOGIN <sup>®</sup> )	VM-restoration	Randomized trial. 117 HPV+BV or VVC-women receiving standard antimicrobial treatment	Probiotic (6 months) resolved HPV-related cytological anomalies twice higher than probiotics during 3 months, induced high total HPV clearance	145
<i>L. rhamnosus</i> HN001	Yoghurt	Oral capsule (Fonterra-Cooperative)	Improvement-maternal health-pregnancy (prevention maternal GDM) and postpartum (depression-anxiety), prevention infant eczema-allergy	Two-center randomized double-blind placebo-controlled trial. 423 pregnant women at 14–16 weeks gestation with personal or partner history of atopic disease and expecting infants at high risk of allergic disease	Probiotic (until delivery and then until 6 months post-partum, if breastfeeding), reduced GDM prevalence, decreased depression and anxiety scores in the postpartum period, did not reduce infant eczema	146–149
<i>L. salivarius</i> CECT 9145	Healthy human vagina	Freeze-dried	GBS-vaginal and rectal colonization-reduction	Prospective pilot clinical trial. 57 pregnant women	Lactobacilli (one/day from week 26/38 pregnancy) reduced rectal and vaginal GBS colonization	150
<i>L. acidophilus</i> La-14, <i>L. rhamnosus</i> HN001	Human feces and yoghurt, respectively	Oral capsule+bovine lactoferrin RCX <sup>™</sup> (Respecta <sup>®</sup> )	VM-improvement Adjunct to BV-therapy	Double-blind randomized placebo-controlled study. 40 healthy women Double-blind placebo-controlled-randomized clinical trial. 48 BV women	Probiotic (twice daily/2 weeks) increased probiotic species in vagina and without adverse effect Probiotic+lactoferrin (2 capsules/day/5 days followed by 1 capsule/day/10 days/month during 6 months) reduced vaginal discharge, itching, nugent score and recurrence rate	151 37
			Adjunct to R-VVC-therapy	Double-blinded placebo-prospective randomized clinical trial. 48 <i>Candida albicans</i> -positive women	Probiotic+lactoferrin (2 capsules/day/5 days followed by 1 capsule/day/10 days/month during 6 months) reduced itching and discharge at 3 and 6 months, and R-VVC	152

(Continues)



TABLE 1 (Continued)

Microorganisms	Isolation site	Pharmaceutical form or product	Clinical target <sup>a</sup>	Type of study and participant type and number	Results	References
<i>L. rhamnosus</i> IMC 501, <i>L. paracasei</i> IMC 502	Elderly human faeces Human feces	Vaginal suppository (SYNBIO <sup>®</sup> )	VM-restoration	Single-arm open-label controlled towards the baseline (pre-post) study. 35 apparently healthy women	Probiotic for 7 days did not produce adverse effects, reduced Nugent score, increased <i>Lactobacillus</i> level, did not modify pH vaginal	140
<i>L. rhamnosus</i> GR-1, <i>L. reuteri</i> RC-14	Healthy human urethra and vagina, respectively	Oral capsule (U-relax <sup>®</sup> )  Oral capsule (Chr. Hansen)	HR-HPV-clearance  VM-modulation	Randomized double-blinded placebo-controlled trial. 121 HR-HPV women  Pilot randomized blinded placebo-controlled trial. 38 pregnant women of gestational age less than 36 weeks	Probiotic (1 capsule/day until negative HR-HPV testing) did not influence HR-HPV clearance, decreased rates of mildly abnormal and unsatisfactory cervical smears  Probiotic (1 capsule/day/1 month) without side effects did not modify VM	153  154
			VM-maintenance/restoration	Randomized placebo-controlled triple-blind parallel group trial. 320 women with <12 completed pregnancy weeks	Probiotic (1 capsule/day/8 weeks) did not modify VM	155
			VM-maintenance/restoration	Randomized double-blind placebo-controlled trial. 304 women with 9-14 pregnancy weeks	Probiotic (1 capsule/day from recruitment until pregnancy end) did not modified VM	156
			GBS-vaginal colonization-reduction	Randomized controlled trial. 99 pregnant women at 35-37 weeks of gestation with vaginal and rectal-GBS positive	Probiotic (2 capsules before bedtime until delivery) reduced the GBS colonization	157
			GBS-vaginal colonization-reduction	Pilot randomized control study. 34 GBS-positive women at 36 weeks pregnant received standard antenatal care	Probiotic (1 capsule/day/3 weeks or until birth) did not reduce vaginal GBS-rates	158
<i>L. fermentum</i> 57A, <i>L. plantarum</i> 57B, <i>L. gasseri</i> 57C	Healthy human vagina	Vaginal capsule (inVag <sup>®</sup> )	VM-restoration	Multicenter randomized double-blind placebo-controlled trial. 160 abnormal VM women	Probiotic (1 capsule/day/7 days) decreased vaginal pH and Nugent score, increased <i>Lactobacillus</i> abundance, without adverse effect	159
		Oral capsule (prOVag <sup>®</sup> )	Adjunct to BV/AV-therapy	Randomized double-blind placebo controlled trial. 154 of recurrent BV/AV histories and current symptoms women.	Probiotic (2 capsule/day/10 days, during follow-up, and one capsule/day/10 days perimenstrually), lengthened time to clinical BV/AV symptoms relapse, reduced and maintained low vaginal pH and Nugent score, increased vaginal <i>Lactobacillus</i> counts	160

(Continues)

TABLE 1 (Continued)

Microorganisms	Isolation site	Pharmaceutical form or product	Clinical target <sup>a</sup>	Type of study and participant type and number	Results	References
<i>L. rhamnosus</i> DSM 14870, <i>L. gasseri</i> DSM 14869	Healthy human vagina	Vaginal capsule (EcoVag®)	Adjunct to BV-therapy	Prospective partially randomized exploratory pilot study. 39 BV women	Probiotic (once/day/30 days then once/week until day 190) colonized vagina, did not improve BV cure rates or alleviate recurrence	161
			Subsequent to BV/R-VVC-therapy	Two pilot open-label clinical trials. 40 Scandinavian BV or VVC-diagnosed women	Probiotic (5 days) induced a 6-month BV cure rate of 50%. Probiotic (10 days after each antibiotic treatment followed by weekly administration of capsules for 4 months) induced 6- and 12-month BV-cure rates of 67%, while the 6- and 12-month VVC-cure rates of 100% and 89%	162
			Adjunct to antibiotic treatment on perinatal outcome with PPROM	Prospective randomized trial. 115 PPROM between 24 and 34 weeks of gestation women	Probiotic (10 days) increased gestational age at birth, duration of latency period and birth weight. Neonates of probiotic-group presented lower chance of entering intensive care unit, shorter total hospitalization time, lower need for oxygen administration and mechanical ventilation, and lower length of oxygen administration	163
<i>L. gasseri</i> LN40, <i>L. rhamnosus</i> LN113, <i>L. fermentum</i> LN99	Healthy human vagina	Intimate care ointment (Ellen AB)	VM-improvement	Double-blind randomized pilot study. 18 healthy postmenopausal women	Probiotic (10 days) induced lactobacilli persistence in vagina for at least 10 days	164
<b>Formula A:</b> <i>L. gasseri</i> CRL1307, CRL1263, <i>L. reuteri</i> CRL1324 <b>Formula B:</b> <i>L. gasseri</i> CRL1256, CRL1320, <i>L. rhamnosus</i> CRL1332	Healthy human vagina	Vaginal capsule	VM-restoration	Double-blind randomized clinical trial of safety. 39 normal or intermediate microbiota women	<i>Lactobacillus</i> formulations (1 capsule/day/7 days) tended to decrease in both Nugent score and vaginal leukocyte number, increased cultivable lactobacilli, without adverse effect	129
<b>Pro-I:</b> <i>L. crispatus</i> EST-1, EST-4, EST-6 <b>Pro-II:</b> <i>L. crispatus</i> EST-2, EST-3, EST-5	Healthy human vagina	Oral capsule	VM-restoration	Randomized double-blind placebo-controlled crossover trial. 40 reproductive-age considered healthy women	Pro-I or Pro-II (once capsule/day/1-week followed by 2-week washout period, continued with second treatment and washout period) were well tolerable, and Pro-II reduced Nugent score and <i>Gardnerella vaginalis</i> counts	165
<b>F1-Probiotic:</b> <i>L. acidophilus</i> PBS066, <i>L. reuteri</i> PBS072 <b>F2-Probiotic:</b> <i>L. plantarum</i> PBS067, <i>L. rhamnosus</i> PBS070, <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> PBS075	Gijg Human feces	Oral capsule+inulin	Recurrent UGTI-prevention	Randomized placebo-controlled pilot study. 60 healthy women	F1 and F2 (14 days) colonized vagina and showed <i>in vitro</i> anti-microbial activity against urogenital pathogens	166

(Continues)

TABLE 1 (Continued)

Microorganisms	Isolation site	Pharmaceutical form or product	Clinical target <sup>a</sup>	Type of study and participant type and number	Results	References
<i>L. crispatus</i> LbV 88, <i>L. gasseri</i> LbV 150 N, <i>L. jensenii</i> LbV 116, <i>L. rhamnosus</i> LbV96	Healthy human vagina	Yoghurt  Oral capsule (Florium, European-Patent-PCT/EP2011/065877)	Adjunct to BV therapy  IM/VM-reconstitution of herpesvirus-pregnant	Double-blind randomized controlled-clinical pilot trial. 36 BV women  Randomized trial. 60 women with herpes virus infection on the 14-16th week of pregnancy	Yoghurt (twice/day/4 weeks) improved BV-recovery rate and symptoms, tended to improve VM  Probiotic (2 capsules/day/30 min before meals/-week) reduced opportunistic pathogens, increased <i>Lactobacillus</i> in intestine and vagina, decreased 2–3 times complaints incidence (bloating/discomfort/constipation/mucus-in-stool/excessive-vaginal-discharge/itching/swelling/redness-of-mucosa), reduced twofold the incidence of placental insufficiency, preeclampsia and fetal distress	167  168
<i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>Streptococcus thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	UD	Vaginal capsule (Lactagyn®)	Subsequent to R-VVC-therapy	Randomized trial. 436 VVC women	Probiotic (10 applications/beginning 5th day after azole treatment) reduced clinical complaints, improved microbiological efficacy	169
<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. lactis</i> , <i>B. bifidum</i> , <i>B. lactis</i>	UD	Oral powder (SimFort; Vitafor Nutrientes)	Adjunct to isoflavone to improve menopause genitourinary symptoms	Randomized trial. 60 postmenopausal-women	Probiotic (one pack) improved isoflavones metabolism after 16 weeks, but failed to yield estrogenic effect on the urogenital tract and relieve vulvovaginal symptoms	170

Abbreviations: AV, aerobic vaginitis; BV, bacterial vaginosis; GBS, Group B *Streptococcus*; GDM, gestational diabetes mellitus; HPV, human papilloma virus; HSV, Herpes simplex virus; IM, intestinal microbiota; PPRM, preterm premature rupture of membranes; TV, trichomoniasis; UD: undetermined; VM, vaginal microbiota; VVC, vulvovaginal candidiasis, R-VVC, recurrent-vulvovaginal candidiasis. Different colors are used to include different women status in the same table. Each color represents a different state: non pregnant (white), pregnant (light gray), and post-menopausal (dark gray).

<sup>a</sup>Products in the market.

TABLE 2 Feminine hygiene products containing vaginal probiotic lactobacilli

Microorganisms	Brand name and type	Claimed effect	References
Lacto Naturel, LN <sup>®</sup> : <i>L. gasseri</i> LN40, <i>L. fermentum</i> LN99, <i>L. casei</i> ssp <i>rhamnosus</i> LN 113, <i>Pediococcus acidilactici</i> LN23	ELLEN <sup>®</sup> probiotic tampon	VM-improvement/restoration	172
	Muvagyn <sup>®</sup> probiotic tampon		
	Florgynal <sup>®</sup> probiotic tampon-Saforelle		
	Natura Femina-Ellen Tampon		
	Ellen <sup>®</sup> LN intimate cleansing foam and intimate grooming/shaving cream	Vaginal pH-maintenance	
	Ellen <sup>®</sup> probiotic intimate topical cream	pH-maintenance, hydration dry mucous	
<i>L. acidophilus</i> pure culture	Natura Femina probiotic paste in cotton sanitary towels	VM and pH-maintenance, reduce discomfort (itching/irritation/discharge/odors), infection or inflammation	173
	Carin/Oasis/Micci ProBiotic ultra wings-sanitary napkins	VM-improvement/restoration	174
	Intimea LACTOPROBIOTIC (probiotics+lactic acid), ultra-thin-dairy use, incontinences	VM-improvement/restoration+lactic acid to prevent infection and vaginal inflammation	175,176

Abbreviation: VM, vaginal microbiota.

LN-bacteria exert their effect exclusively in the vaginal cavity and on/in the mucus covering the squamous vaginal epithelial cells. The release of probiotic bacteria and the production of lactic acid are the main proposed effects.

A group of experts from the Polish Gynecological Society evaluated the available bibliography and issued a statement concluding that this product is an innovative solution allowing the vaginal application of strains with beneficial probiotic properties during menstrual bleeding.<sup>177</sup> Sauperl et al.<sup>173</sup> have later proposed the incorporation of a probiotic paste with the same LN strains to the surface of sanitary napkins in the design of functionalized sanitary products.

Other sets of products are Carin/Oasis/Micci ProBiotic, containing *L. acidophilus* in the inner area of feminine sanitary napkins, while Intimea LACTOPROBIOTIC feminine pads include *L. acidophilus* on the surface, registered in United States and Europe patents.<sup>175,176</sup> Dried lactobacilli are added through contact sorption drying carriers in a lipid phase. The isolation source of these strains neither their effect is unknown.

On the other hand, nanotechnology applying the electrospinning technique has recently been proposed to cover different devices for vaginal uses with viable *Lactobacillus* in a single step.<sup>178,179</sup> The coating system was successfully applied in several products, as stent, soybeans, cellulose paper, with a wide variety of bioactive immobilized in nanofibers through electrospinning.<sup>180-182</sup> Then, this *Lactobacillus* immobilization system offers advantages when compared with lyophilized powders, since modifications of the final product are not evidenced, and subsequent processing for their incorporation in the product design are not required. There are few publications on the application of probiotics immobilized in nanofibers for vaginal application available. Škrlec et al.<sup>183</sup> immobilized probiotic *L. plantarum* ATCC 8014 (unknown origin) in

nanofibers prolonging the strain viability. In the case of vaginal strains, Nagy et al.<sup>184</sup> immobilized *L. acidophilus* in polyvinylalcohol and polyvinylpyrrolidone nanofibers, suggesting them for BV therapy, but did not prove their efficacy. The immobilization of a *L. rhamnosus* CRL 1332 vaginal strain in polyvinylalcohol nanofibers by electrospinning was successfully, maintaining the required viable cell numbers during 360 days at 4°C and the inhibition to urogenital pathogens.<sup>178</sup> Up to date, clinical trials to determine the safety, efficacy and effectiveness of immobilized probiotics in these products for UGTI treatment were not published.

## 8 | CONCLUSIONS

*Lactobacillus* species are the predominant microorganisms in the healthy human vaginal microbiome, and their inclusion as probiotic for the UGT in medical clinical practice is widely recommended. Evidence of their multiple and potential effects must be demonstrated, mainly referred to the VM reestablishment and to the preventive/therapeutic effect against UGTI. Urogenital probiotics designed with different bacterial strains, doses, treatment schemes, routes, and vehicles of administration were clinically evaluated. The application in the preterm birth area, in the improvement of maternal health pregnancy and postpartum, and prevention of infant eczema-allergy must be encouraged. A promising area is the probiotic inclusion in daily use-hygiene products with nanofiber-immobilized lactobacilli, with interesting application possibilities. The potential benefits of probiotics use on the health of women around the world strongly support the requirement of further studies to complement the current knowledge and to encourage clinical applications of probiotics in the UGT, either as preventive or therapeutic agents.


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## DISCLOSURE

The authors declare there is no conflict of interest. Some of the results of the research group were included in a patent presentation (INPI, 2018, N° 20180103893).

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## REFERENCES

- Human Microbiome Project Consortium. A framework for human microbiome research. *Nature*. 2012;486(7402):215-221. <https://doi.org/10.1038/nature11209>.
- Verhelst R, Verstraelen H, Claeys G, et al. Comparison between Gram stain and culture for the characterization of vaginal microflora: definition of a distinct grade that resembles grade I microflora and revised categorization of grade I microflora. *BMC Microbiol*. 2005;5:61. <https://doi.org/10.1186/1471-2180-5-61>.
- Zhou X, Hansmann MA, Davis CC, et al. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. *FEMS Immunol Med Microbiol*. 2010;58(2):169-181. <https://doi.org/10.1111/j.1574-695X.2009.00618.x>.
- Sroka-Oleksiak A, Gosiewski T, Pabian W, et al. Next-generation sequencing as a tool to detect vaginal microbiota disturbances during pregnancy. *Microorganisms*. 2020;8(11):1813. <https://doi.org/10.3390/microorganisms8111813>.
- Fettweis JM, Serrano MG, Girerd PH, Jefferson KK, Buck GA. A new era of the vaginal microbiome: advances using next-generation sequencing. *Chem Biodivers*. 2012;9(5):965-976. <https://doi.org/10.1002/cbdv.201100359>.
- Jespers V, Menten J, Smet H, et al. Quantification of bacterial species of the vaginal microbiome in different groups of women, using nucleic acid amplification tests. *BMC Microbiol*. 2012;12(1):83. <https://doi.org/10.1186/1471-2180-12-83>.
- Datcu R, Gesink D, Mulvad G, et al. Vaginal microbiome in women from Greenland assessed by microscopy and quantitative PCR. *BMC Infect Dis*. 2013;13(1):480. <https://doi.org/10.1186/1471-2334-13-480>.
- Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A*. 2011;1:4680-4687. <https://doi.org/10.1073/pnas.1002611107>.
- Martin DH. The microbiota of the vagina and its influence on women's health and disease. *Am J Med Sci*. 2012;343(1):2-9. <https://doi.org/10.1097/MAJ.0b013e31823ea228>.
- Hummelen R, Fernandes AD, Macklaim JM, et al. Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One*. 2010;5(8):e12078. <https://doi.org/10.1371/journal.pone.0012078>.
- Forney LJ, Gajer P, Williams CJ, et al. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol*. 2010;48(5):1741-1748. <https://doi.org/10.1128/JCM.01710-09>.
- Drell T, Lillsaar T, Tummeleht L, et al. Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age estonian women. *PLoS One*. 2013;8(1):e54379-<https://doi.org/10.1371/journal.pone.0054379>
- Sharma A, Lee S, Park YS. Molecular typing tools for identifying and characterizing lactic acid bacteria: a review. *Food Sci Biotechnol*. 2020;29(10):1301-1318. <https://doi.org/10.1007/s10068-020-00802-x>.
- Gupta S, Kakkar V, Bhushan I. Crosstalk between vaginal microbiome and female health: a review. *Microb Pathog*. 2019;136:103696. <https://doi.org/10.1016/j.micpath.2019.103696>.
- Buchta V. Vaginal microbiome. *Vaginální mikrobiom. Ceska Gynekol*. 2018;83(5):371-379.
- Hunter CA, Long KR, Schumacher RR. A study of döderlein's vaginal bacillus\*. *Ann N Y Acad Sci*. 1959;83(2):217-226. <https://doi.org/10.1111/j.1749-6632.1960.tb40894.x>.
- He Y, Huang Y, Zhang Z, et al. Exploring profile and potential influencers of vaginal microbiome among asymptomatic pregnant Chinese women. *PeerJ*. 2019;7:e8172. <https://doi.org/10.7717/peerj.8172>.
- Delgado-Diaz DJ, Tyssen D, Hayward JA, et al. Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota. *Front Cell Infect Microbiol*. 2020;9:446. <https://doi.org/10.3389/fcimb.2019.00446>.
- Hočevár K, Maver A, Vidmar Šimic M, et al. Vaginal microbiome signature is associated with spontaneous preterm delivery. *Front Med*. 2019;6:201. <https://doi.org/10.3389/fmed.2019.00201>.
- Ma ZS, Li L. Quantifying the human vaginal community state types (CSTs) with the species specificity index. *PeerJ*. 2017;2017(5):e3366. <https://doi.org/10.7717/peerj.3366>.
- Proctor LM, Creasy HH, Fettweis JM, et al. The integrative human microbiome project. *Nature*. 2019;569(7758):641-648. <https://doi.org/10.1038/s41586-019-1238-8>.
- Power ML, Quagliari C, Schulkin J. Reproductive microbiomes: a new thread in the microbial network. *Reprod Sci*. 2017;24(11):1482-1492. <https://doi.org/10.1177/1933719117698577>.
- De Agüero MG, Ganal-Vonarburg SC, Fuhrer T, et al. The maternal microbiota drives early postnatal innate immune development. *Science*. 2016;351(6279):1296-1302. <https://doi.org/10.1126/science.aad2571>.
- Romano-Keeler J, Weitkamp JH. Maternal influences on fetal microbial colonization and immune development. *Pediatr Res*. 2015;77(1-2):189-195. <https://doi.org/10.1038/pr.2014.163>.
- Kamińska D, Gajecka M. Is the role of human female reproductive tract microbiota underestimated? *Benef Microbes*. 2017;8(3):327-343. <https://doi.org/10.3920/BM2015.0174>.
- Wee BA, Thomas M, Sweeney EL, et al. A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Aust New Zeal J Obstet Gynaecol*. 2018;58(3):341-348. <https://doi.org/10.1111/ajo.12754>.
- Moreno I, Simon C. Relevance of assessing the uterine microbiota in infertility. *Fertil Steril*. 2018;110(3):337-343. <https://doi.org/10.1016/j.fertnstert.2018.04.041>.
- Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. Role of the microbiome in human development. *Gut*. 2019;68(6):1108-1114. <https://doi.org/10.1136/gutjnl-2018-317503>.
- Nagpal R, Tsuji H, Takahashi T, et al. Sensitive quantitative analysis of the meconium bacterial microbiota in healthy term infants born vaginally or by cesarean section. *Front Microbiol*. 2016;7:1997. <https://doi.org/10.3389/fmicb.2016.01997>.
- Stearns JC, Simioni J, Gunn E, et al. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. *Sci Rep*. 2017;7(1):1-9. <https://doi.org/10.1038/s41598-017-16606-9>.
- Korpela K, Costea P, Coelho LP, et al. Selective maternal seeding and environment shape the human gut microbiome.

- Genome Res.* 2018;28(4):561-568. <https://doi.org/10.1101/gr.233940.117>.
32. Aragón IM, Herrera-Imbroda B, Queipo-Ortuño MI, et al. The urinary tract microbiome in health and disease. *Eur Urol Focus.* 2018;4(1):128-138. <https://doi.org/10.1016/j.euf.2016.11.001>.
  33. Fuochi V, Volti GL, Furneri PM. Commentary: Lactobacilli dominance and vaginal pH: why is the human vaginal microbiome unique? *Front Microbiol.* 2017;8:1815. <https://doi.org/10.3389/fmicb.2017.01815>.
  34. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. *Res Microbiol.* 2017;168(9-10):782-792. <https://doi.org/10.1016/j.resmic.2017.04.001>.
  35. Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol.* 2019;18(1):40-50. <https://doi.org/10.1002/rmb2.12249>.
  36. Eschenbach DA, Thwin SS, Patton DL, et al. Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clin Infect Dis.* 2000;30(6):901-907. <https://doi.org/10.1086/313818>.
  37. Russo R, Karadja E, De Seta F. Evidence-based mixture containing *Lactobacillus* strains and lactoferrin to prevent recurrent bacterial vaginosis: a double blind, placebo controlled, randomised clinical trial. *Benef Microbes.* 2019;10(1):19-26. <https://doi.org/10.3920/BM2018.0075>.
  38. Nunn KL, Forney LJ. Unraveling the dynamics of the human vaginal microbiome. *Yale J Biol Med.* 2016;89(3):331-337. <https://pubmed.ncbi.nlm.nih.gov/27698617/>. Accessed October 29, 2020.
  39. Bosch TCG. Rethinking the role of immunity: lessons from hydra. *Trends Immunol.* 2014;35(10):495-502. <https://doi.org/10.1016/j.it.2014.07.008>.
  40. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. *Hum Reprod Update.* 2015;21(3):353-377. <https://doi.org/10.1093/humupd/dmu065>.
  41. Nasu K, Narahara H. Pattern recognition via the toll-like receptor system in the human female genital tract. *Mediators Inflamm.* 2010;2010:976024. <https://doi.org/10.1155/2010/976024>.
  42. Kalia N, Singh J, Kaur M. Immunopathology of recurrent vulvovaginal infections: new aspects and research directions. *Front Immunol.* 2019;10:2034. <https://doi.org/10.3389/fimmu.2019.02034>.
  43. Miller-Ensminger T, Mormando R, Maskeri L, Shapiro JW, Wolfheid AJ, Putontiid C. Introducing Lu-1, a novel *Lactobacillus jensenii* phage abundant in the urogenital tract. *PLoS One.* 2020;15(6):e0234159. <https://doi.org/10.1371/journal.pone.0234159>.
  44. Kiliç AO, Pavlova SI, Alpaly S, Kiliç SS, Tao L. Comparative study of vaginal *Lactobacillus* phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology. *Clin Diagn Lab Immunol.* 2001;8(1):31-39. <https://doi.org/10.1128/CDLI.8.1.31-39.2001>.
  45. Zhao W, Hao M, Wang Y, et al. Association between folate status and cervical intraepithelial neoplasia. *Eur J Clin Nutr.* 2016;70(7):837-842. <https://doi.org/10.1038/ejcn.2016.35>.
  46. Siegel EM, Salemi JL, Villa LL, Ferenczy A, Franco EL, Giuliano AR. Dietary consumption of antioxidant nutrients and risk of incident cervical intraepithelial neoplasia. *Gynecol Oncol.* 2010;118(3):289-294. <https://doi.org/10.1016/j.ygyno.2010.05.022>.
  47. Seo SS, Oh HY, Lee JK, Kong JS, Lee DO, Kim MK. Combined effect of diet and cervical microbiome on the risk of cervical intraepithelial neoplasia. *Clin Nutr.* 2016;35(6):1434-1441. <https://doi.org/10.1016/j.clnu.2016.03.019>.
  48. Brooks JP, Edwards DJ, Blithe DL, et al. Effects of combined oral contraceptives, depot medroxyprogesterone acetate and the levonorgestrel-releasing intrauterine system on the vaginal microbiome. *Contraception.* 2017;95(4):405-413. <https://doi.org/10.1016/j.contraception.2016.11.006>.
  49. Noyes N, Cho K-C, Ravel J, Forney LJ, Abdo Z. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS One.* 2018;13(1):e0191625. <https://doi.org/10.1371/journal.pone.0191625>.
  50. Fashemi B, Delaney ML, Onderdonk AB, Fichorova RN. Effects of feminine hygiene products on the vaginal mucosal biome. *Microb Ecol Heal Dis.* 2013;24: <https://doi.org/10.3402/mehd.v24i0.19703>.
  51. Amabebe E, Anumba DOC. Psychosocial stress, cortisol levels, and maintenance of vaginal health. *Front Endocrinol.* 2018;9:568. <https://doi.org/10.3389/fendo.2018.00568>.
  52. Lewis FMT, Bernstein KT, Aral SO. Vaginal microbiome and its relationship to behavior, sexual health, and sexually transmitted diseases. *Obstet Gynecol.* 2017;129(4):643-654. <https://doi.org/10.1097/AOG.0000000000001932>.
  53. Nogacka A, Salazar N, Suárez M, et al. Impact of intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. *Microbiome.* 2017;5(1):93. <https://doi.org/10.1186/s40168-017-0313-3>.
  54. Mei C, Yang W, Wei X, Wu K, Huang D. The unique microbiome and innate immunity during pregnancy. *Front Immunol.* 2019;10:2886. <https://doi.org/10.3389/fimmu.2019.02886>.
  55. Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S. *Lactobacillus* species as biomarkers and agents that can promote various aspects of vaginal health. *Front Physiol.* 2015;6:81. <https://doi.org/10.3389/fphys.2015.00081>.
  56. Bajic P, Wolfe AJ, Gupta GN. The urinary microbiome: implications in bladder cancer pathogenesis and therapeutics. *Urology.* 2019;126:10-15. <https://doi.org/10.1016/j.urology.2018.12.034>.
  57. Stapleton AE. The vaginal microbiota and urinary tract infection. *Microbiol Spectr.* 2016;4(6). <https://doi.org/10.1128/microbiolspec.uti-0025-2016>.
  58. Gupta V, Nag D, Garg P. Recurrent urinary tract infections in women: How promising is the use of probiotics? *Indian J Med Microbiol.* 2017;35(3):347-354. [https://doi.org/10.4103/ijmm.IJMM\\_16\\_292](https://doi.org/10.4103/ijmm.IJMM_16_292).
  59. Wagenlehner F, Wullt B, Ballarini S, Zingg D, Naber KG. Social and economic burden of recurrent urinary tract infections and quality of life: a patient web-based study (GESPRIT). *Expert Rev Pharmacoecon Outcomes Res.* 2018;18(1):107-117. <https://doi.org/10.1080/14737167.2017.1359543>.
  60. Foxman B. Urinary tract infection syndromes. Occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am.* 2014;28(1):1-13. <https://doi.org/10.1016/j.idc.2013.09.003>.
  61. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against uropathogenic *Escherichia coli*. *Mol Immunol.* 2019;108:56-67. <https://doi.org/10.1016/j.molimm.2019.02.007>.
  62. Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Microbiol Spectr.* 2016;4(2): <https://doi.org/10.1128/microbiolspec.uti-0012-2012>.
  63. Renard J, Ballarini S, Mascarenhas T, et al. Recurrent lower urinary tract infections have a detrimental effect on patient quality of life: a prospective, observational study. *Infect Dis Ther.* 2015;4(1):125-135. <https://doi.org/10.1007/s40121-014-0054-6>.
  64. Kirjavainen PV, Pautler S, Baroja ML, et al. Abnormal immunological profile and vaginal microbiota in women prone to urinary tract infections. *Clin Vaccine Immunol.* 2009;16(1):29-36. <https://doi.org/10.1128/CVI.00323-08>.
  65. Muhleisen AL, Herbst-Kralovetz MM. Menopause and the vaginal microbiome. *Maturitas.* 2016;91:42-50. <https://doi.org/10.1016/j.maturitas.2016.05.015>.

66. Sihra N, Goodman A, Zakri R, Sahai A, Malde S. Nonantibiotic prevention and management of recurrent urinary tract infection. *Nat Rev Urol*. 2018;15(12):750-776. <https://doi.org/10.1038/s41585-018-0106-x>.
67. Singh S, Verma N, Taneja N. The human gut resistome: current concepts & future prospects. *Indian J Med Res*. 2019;150(4):345-358. [https://doi.org/10.4103/ijmr.IJMR\\_1979\\_17](https://doi.org/10.4103/ijmr.IJMR_1979_17).
68. Lanza VF, Baquero F, Martínez JL, et al. In-depth resistome analysis by targeted metagenomics. *Microbiome*. 2018;6(1):11. <https://doi.org/10.1186/s40168-017-0387-y>.
69. ISAPP. *Probiotics: A Consumer Guide for Making Smart Choices Developed by the International Scientific Association for Probiotics and Prebiotics*. International Scientific Association for Probiotics and Prebiotics; 2013. <http://www.isapp.net>. Accessed January 25, 2021.
70. Hill C, Guarner F, Reid G, et al. Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514. <https://doi.org/10.1038/nrgastro.2014.66>.
71. Lopez M, Li N, Kataria J, Russell M, Neu J. Live and ultraviolet-inactivated *Lactobacillus rhamnosus* GG decrease flagellin-induced interleukin-8 production in Caco-2 cells. *J Nutr*. 2008;138(11):2264-2268. <https://doi.org/10.3945/jn.108.093658>.
72. Ostad SN, Salarian AA, Ghahramani MH, Fazeli MR, Samadi N, Jamalifar H. Live and heat-inactivated lactobacilli from feces inhibit *Salmonella typhi* and *Escherichia coli* adherence to Caco-2 cells. *Folia Microbiol*. 2009;54(2):157-160. <https://doi.org/10.1007/s12223-009-0024-7>.
73. Rampengan NH, Manoppo J, Warouw SM. Comparison of efficacies between live and killed probiotics in children with lactose malabsorption. *Southeast Asian J Trop Med Public Health*. 2010;41(2):474-481.
74. Taverniti V, Guglielmetti S. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). *Genes Nutr*. 2011;6(3):261-274. <https://doi.org/10.1007/s12263-011-0218-x>.
75. Tsilingiri K, Rescigno M. Postbiotics: what else? *Benef Microbes*. 2013;4(1):101-107. <https://doi.org/10.3920/BM2012.0046>.
76. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, et al. Postbiotics: an evolving term within the functional foods field. *Trends Food Sci Technol*. 2018;75:105-114. <https://doi.org/10.1016/j.tifs.2018.03.009>.
77. Żótkiewicz J, Marzec A, Ruszczynski M, Feleszko W. Postbiotics-A step beyond pre- and probiotics. *Nutrients*. 2020;12(8):2189. <https://doi.org/10.3390/nu12082189>.
78. Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE. Postbiotics and paraprobiotics: from concepts to applications. *Food Res Int*. 2020;136:109502. <https://doi.org/10.1016/j.foodres.2020.109502>.
79. Zendeboodi F, Khorshidian N, Mortazavian AM, da Cruz AG. Probiotic: conceptualization from a new approach. *Curr Opin Food Sci*. 2020;32:103-123. <https://doi.org/10.1016/j.cofs.2020.03.009>.
80. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics—a review. *J Food Sci Technol*. 2015;52(12):7577-7587. <https://doi.org/10.1007/s13197-015-1921-1>.
81. Fu N, Huang S, Xiao J, Chen XD. Producing powders containing active dry probiotics with the aid of spray drying. *Adv Food Nutr Res*. 2018;85:211-262. <https://doi.org/10.1016/bs.afnr.2018.02.003>.
82. Champagne CP, da Cruz AG, Daga M. Strategies to improve the functionality of probiotics in supplements and foods. *Curr Opin Food Sci*. 2018;22:160-166.
83. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep*. 2006;7(7):688-693. <https://doi.org/10.1038/sj.embor.7400731>.
84. LeBegue CE, Love BL, Wyatt MD. Microbes as drugs: the potential of pharmabiotics. *Pharmacotherapy*. 2020;40(2):102-106. <https://doi.org/10.1002/phar.2357>.
85. Cordaillat-Simmons M, Rouanet A, Pot B. Live biotherapeutic products: the importance of a defined regulatory framework. *Exp Mol Med*. 2020;52(9):1397-1406. <https://doi.org/10.1038/s12276-020-0437-6>.
86. Nader-Macías MEF, Juárez Tomás MS. Profiles and technological requirements of urogenital probiotics. *Adv Drug Deliv Rev*. 2015;92:84-104. <https://doi.org/10.1016/j.addr.2015.03.016>.
87. Salvetti E, O'Toole PW. The genomic basis of lactobacilli as health-promoting organisms. In: *Bugs as Drugs*. Vol 5. American Society of Microbiology; 2017:49-71. <https://doi.org/10.1128/microbiolspec.bad-0011-2016>.
88. McFarland LV, Evans CT, Goldstein EJC. Strain-specificity and disease-specificity of probiotic efficacy: a systematic review and meta-analysis. *Front Med*. 2018;5:124. <https://doi.org/10.3389/fmed.2018.00124>.
89. Pan M, Hidalgo-Cantabrana C, Goh YJ, Sanozky-Dawes R, Barrangou R. Comparative analysis of *Lactobacillus gasseri* and *Lactobacillus crispatus* isolated from human urogenital and gastrointestinal tracts. *Front Microbiol*. 2020;10:3146. <https://doi.org/10.3389/fmicb.2019.03146>.
90. Yildirim S, Yeoman C, Janga S, et al. Primate vaginal microbiomes exhibit species specificity without universal *Lactobacillus* dominance. *ISME J*. 2014;8:2431-2444. <https://doi.org/10.1038/ismej.2014.90>.
91. Mendes-Soares H, Suzuki H, Hickey RJ, Forney LJ. Comparative functional genomics of *Lactobacillus* spp. reveals possible mechanisms for specialization of vaginal lactobacilli to their environment. *J Bacteriol*. 2014;196(7):1458-1470. <https://doi.org/10.1128/JB.01439-13>.
92. Van Der Veer C, Hertzberger RY, Bruisten SM, et al. Comparative genomics of human *Lactobacillus crispatus* isolates reveals genes for glycosylation and glycogen degradation: Implications for in vivo dominance of the vaginal microbiota. *Microbiome*. 2019;7(1):49. <https://doi.org/10.1186/s40168-019-0667-9>.
93. FAO/WHO. *Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food*. London, ON: FAO/WHO; 2002.
94. Reid G. The development of probiotics for women's health. *Can J Microbiol*. 2017;63(4):269-277. <https://doi.org/10.1139/cjm-2016-0733>.
95. European Food Safety Authority. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA J*. 2007;5(12):1-16. <https://doi.org/10.2903/j.efsa.2007.587>.
96. Alayande KA, Aiyegoro OA, Nengwekhulu TM, Katata-Seru L, Ateba CN. Integrated genome-based probiotic relevance and safety evaluation of *Lactobacillus reuteri* PNW1. *PLoS One*. 2020;15(7):e0235873. <https://doi.org/10.1371/journal.pone.0235873>.
97. van Reenen CA, Dicks LM. Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. *Arch Microbiol*. 2011;193(3):157-168.
98. Gueimonde M, Sánchez B, de Los Reyes-Gavilán GC, Margolles A. Antibiotic resistance in probiotic bacteria. *Front Microbiol*. 2013;4:202. <https://doi.org/10.3389/fmicb.2013.00202>.
99. Imperial IC, Ibane JA. Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. *Front Microbiol*. 2016;7:1983. <https://doi.org/10.3389/fmicb.2016.01983>.
100. von Wintersdorff CJ, Penders J, van Niekerk JM, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol*. 2016;7:173. <https://doi.org/10.3389/fmicb.2016.00173>.

101. Daniali M, Nikfar S, Abdollahi M. Antibiotic resistance propagation through probiotics. *Expert Opin Drug Metab Toxicol.* 2020;16(12):1207-1215. <https://doi.org/10.1080/17425255.2020.1825682>.
102. Sharma K, Attri S, Goel G. Selection and evaluation of probiotic and functional characteristics of autochthonous lactic acid bacteria isolated from fermented wheat flour dough babroo. *Probiotics Antimicrob Proteins.* 2019;11(3):774-784. <https://doi.org/10.1007/s12602-018-9466-z>.
103. EFSA. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. *EFSA J.* 2017;16(1):5131.
104. Wojnicz D, Tichaczek-Goska D, Korzekwa K, Kicia M, Hendrich AB. Study of the impact of cranberry extract on the virulence factors and biofilm formation by *Enterococcus faecalis* strains isolated from urinary tract infections. *Int J Food Sci Nutr.* 2016;67(8):1005-1016. <https://doi.org/10.1080/09637486.2016.1211996>.
105. Granato PA. The microbiota of humans and microbial virulence factors. In: *Biological Safety: Principles and Practices.* 2017:1-17. <https://doi.org/10.1128/9781555819637.ch1>.
106. Touret T, Oliveira M, Semedo-Lemsaddek T. Putative probiotic lactic acid bacteria isolated from sauerkraut fermentations. *PLoS One.* 2018;13(9):e0203501. <https://doi.org/10.1371/journal.pone.0203501>.
107. Hernández-García JE, Sebastián-Frizzo L, Rodríguez-Fernández JC, et al. Evaluación in vitro del potencial probiótico de *Lactobacillus acidophilus* SS80 y *Streptococcus thermophilus* SS77. *Rev Salud Anim.* 2019;41(1):e09.
108. Yao XY, Yuan MM, Li DJ. Molecular adjuvant C3d3 improved the anti-hCGbeta humoral immune response in vaginal inoculation with live recombinant *Lactobacillus* expressing hCGbeta-C3d3 fusion protein. *Vaccine.* 2007;25(32):6129-6139. <https://doi.org/10.1016/j.vaccine.2007.04.090>.
109. Saksena S, Goyal S, Raheja G, et al. Upregulation of P-glycoprotein by probiotics in intestinal epithelial cells and in the dextran sulfate sodium model of colitis in mice. *Am J Physiol Gastrointest Liver Physiol.* 2011;300(6):G1115-G1123. <https://doi.org/10.1152/ajpgi.00027.2011>.
110. Ballini A, Santacroce L, Cantore S, et al. Probiotics improve urogenital health in women. *Open Access Maced J Med Sci.* 2018;6(10):1845-1850. <https://doi.org/10.3889/oamjms.2018.406>.
111. Patrignani F, Siroli L, Parolin C, Serrazanetti DI, Vitali B, Lanciotti R. Use of *Lactobacillus crispatus* to produce a probiotic cheese as potential gender food for preventing gynaecological infections. *PLoS One.* 2019;14(1):e0208906. <https://doi.org/10.1371/journal.pone.0208906>.
112. Lagenaur LA, Lee PP, Hamer DH, Sanders-Beer BE. Demonstration of vaginal colonization with GusA-expressing *Lactobacillus jensenii* following oral delivery in rhesus macaques. *Res Microbiol.* 2011;162(10):1006-1010. <https://doi.org/10.1016/j.resmic.2011.08.003>.
113. Reid G. Has knowledge of the vaginal microbiome altered approaches to health and disease? *F1000Res.* 2018;7:460. <https://doi.org/10.12688/f1000research.13706.1>.
114. Buggio L, Somigliana E, Borghi A, Vercellini P. Probiotics and vaginal microecology: fact or fancy? *BMC Womens Health.* 2019;19(1): <https://doi.org/10.1186/s12905-019-0723-4>.
115. De Araújo Pereira RR, Bruschi ML. Vaginal mucoadhesive drug delivery systems. *Drug Dev Ind Pharm.* 2012;38(6):643-652. <https://doi.org/10.3109/03639045.2011.623355>.
116. Baloglu E, Senyigit ZA, Karavana SY, Bernkop-Schnürch A. Strategies to prolong the intravaginal residence time of drug delivery systems. *J Pharm Pharm Sci.* 2009;12(3):312-336. <https://doi.org/10.18433/j3hp41>.
117. Dobaría N, Mashru R, Vadia NH. Vaginal drug delivery systems: a review of current status. *East Cent African J Pharm Sci.* 2008;10(1):3-13. <https://doi.org/10.4314/ecajps.v10i1.9754>.
118. Sahoo CK, Kumar Nayak P, Sarangi DK, Sahoo TK. Intra vaginal drug delivery system: an overview. *Am J Adv Drug Deliv.* 2013;2013:43-55. [www.ajadd.co.uk](http://www.ajadd.co.uk). Accessed October 29, 2020.
119. Juárez Tomás MS, Bru E, Martos G, Nader-Macías ME. Stability of freeze-dried vaginal *Lactobacillus* strains in the presence of different lyoprotectors. *Can J Microbiol.* 2009;55(5):544-552. <https://doi.org/10.1139/w08-159>.
120. Vitali B, Abruzzo A, Parolin C, et al. Association of *Lactobacillus crispatus* with fructo-oligosaccharides and ascorbic acid in hydroxypropyl methylcellulose vaginal insert. *Carbohydr Polym.* 2016;136:1161-1169. <https://doi.org/10.1016/j.carbpol.2015.10.035>.
121. Borges S, Costa P, Silva J, Teixeira P. Effects of processing and storage on *Pediococcus pentosaceus* SB83 in vaginal formulations: lyophilized powder and tablets. *Biomed Res Int.* 2013;2013:680767. <https://doi.org/10.1155/2013/680767>.
122. Juárez Tomás MS, De Gregorio PR, Leccese Terraf MC, Nader-Macías MEF. Encapsulation and subsequent freeze-drying of *Lactobacillus reuteri* CRL 1324 for its potential inclusion in vaginal probiotic formulations. *Eur J Pharm Sci.* 2015;79:87-95. <https://doi.org/10.1016/j.ejps.2015.08.010>.
123. Rohan LC, Sassi AB. Vaginal drug delivery systems for HIV prevention. *AAPS J.* 2009;11(1):78-87. <https://doi.org/10.1208/s12248-009-9082-7>.
124. Nader-Macías ME. Probiotics in the prevention of urogenital tract infections. Mechanisms involved. *Curr Womens Health Rev.* 2006;2(2):103-117. <https://doi.org/10.2174/157340406776931025>.
125. Leccese Terraf MC, Juárez Tomás MS, Nader-Macías ME, Silva C. Screening of biofilm formation by beneficial vaginal lactobacilli and influence of culture media components. *J Appl Microbiol.* 2012;113(6):1517-1529. <https://doi.org/10.1111/j.1365-2672.2012.05429.x>.
126. Leccese Terraf MC, Mendoza LM, Juárez Tomás MS, et al. Phenotypic surface properties (aggregation, adhesion and biofilm formation) and presence of related genes in beneficial vaginal lactobacilli. *J Appl Microbiol.* 2014;117(6):1761-1772. <https://doi.org/10.1111/jam.12642>.
127. Leccese Terraf MC, Juárez Tomás MS, Rault L, et al. In vitro effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic *Escherichia coli*. *Arch Microbiol.* 2017;199(5):767-774. <https://doi.org/10.1007/s00203-016-1336-z>.
128. Reid G. Probiotics: definition, scope and mechanisms of action. *Best Pract Res Clin Gastroenterol.* 2016;30(1):17-25. <https://doi.org/10.1016/j.bpg.2015.12.001>.
129. De Gregorio PR, Maldonado NC, Pingitore EV, et al. Intravaginal administration of gelatine capsules containing freeze-dried autochthonous lactobacilli: a double-blind, randomised clinical trial of safety. *Benef Microbes.* 2020;11(1):5-17. <https://doi.org/10.3920/BM2019.0081>.
130. De Gregorio PR, Tomás MSJ, Terraf MCL, Nader-Macías MEF. In vitro and in vivo effects of beneficial vaginal lactobacilli on pathogens responsible for urogenital tract infections. *J Med Microbiol.* 2014;63(Pt 5):685-696. <https://doi.org/10.1099/jmm.0.069401-0>.
131. De Gregorio PR, Juárez Tomás MS, Leccese Terraf MC, Nader-Macías MEF. Preventive effect of *Lactobacillus reuteri* CRL 1324 on group B *Streptococcus* vaginal colonization in an experimental mouse model. *J. Appl. Microbiol.* 2015;118(4):1034-1047. <https://doi.org/10.1111/jam.12739>.
132. De Gregorio PR, Juárez Tomás MS, Nader-Macías ME. Immunomodulation of *Lactobacillus reuteri* CRL1324 on group B *Streptococcus* vaginal colonization in a murine experimental model.



- Am J Reprod Immunol.* 2016;75(1):23-35. <https://doi.org/10.1111/aji.12445>.
133. Mastromarino P, Vitali B, Mosca L. Bacterial vaginosis: a review on clinical trials with probiotics. *New Microbiol.* 2013;36(3):229-238.
  134. Merenstein D, Guzzi J, Sanders ME. More information needed on probiotic supplement product labels. *J Gen Intern Med.* 2019;34(12):2735-2737. <https://doi.org/10.1007/s11606-019-05077-5>.
  135. Galdeano CM, Perdígón G. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J Appl Microbiol.* 2004;97(4):673-681. <https://doi.org/10.1111/j.1365-2672.2004.02353.x>.
  136. Cazorla SI, Maldonado-Galdeano C, Weill R, De Paula J, Perdígón GDV. Oral administration of probiotics increases paneth cells and intestinal antimicrobial activity. *Front Microbiol.* 2018;9:736. <https://doi.org/10.3389/fmicb.2018.00736>.
  137. Kang CH, Kim Y, Han SH, Kim JS, Paek NS, So JS. In vitro probiotic properties of vaginal *Lactobacillus fermentum* MG901 and *Lactobacillus plantarum* MG989 against *Candida albicans*. *Eur J Obstet Gynecol Reprod Biol.* 2018;228:232-237. <https://doi.org/10.1016/j.ejogrb.2018.07.005>.
  138. de Vrese M, Laue C, Papazova E, Petricevic L, Schrezenmeir J. Impact of oral administration of four *Lactobacillus* strains on Nugent score—systematic review and meta-analysis. *Benef Microbes.* 2019;10(5):483-496. <https://doi.org/10.3920/BM2018.0129>.
  139. Vladareanu R, Mihu D, Mitran M, et al. New evidence on oral *L. plantarum* P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): a randomized double-blind placebo-controlled study. *Eur Rev Med Pharmacol Sci.* 2018;22:262-267. [https://doi.org/10.26355/eurrev\\_201801\\_14128](https://doi.org/10.26355/eurrev_201801_14128).
  140. Verdenelli MC, Cecchini C, Coman MM, et al. Impact of probiotic SYN BIO<sup>®</sup> administered by vaginal suppositories in promoting vaginal health of apparently healthy women. *Curr Microbiol.* 2016;73(4):483-490. <https://doi.org/10.1007/s00284-016-1085-x>.
  141. Cohen CR, Wierzbicki MR, French AL, et al. Randomized trial of lactin-V to prevent recurrence of bacterial vaginosis. *N Engl J Med.* 2020;382(20):1906-1915. <https://doi.org/10.1056/NEJMo a1915254>.
  142. Nader-Macias M, de Ruiz C, Ocana V, Juarez TM. Advances in the knowledge and clinical applications of lactic acid bacteria as probiotics in the urogenital tract. *Curr Womens Health Rev.* 2008;4(4):240-257. <https://doi.org/10.2174/157340408786848214>.
  143. Dausset C, Patrier S, Gajer P, et al. Comparative phase I randomized open-label pilot clinical trial of Gynophilus<sup>®</sup> (Lcr regenerans<sup>®</sup>) immediate release capsules versus slow release muco-adhesive tablets. *Eur J Clin Microbiol Infect Dis.* 2018;37(10):1869-1880. <https://doi.org/10.1007/s10096-018-3321-8>.
  144. Sgibnev AKE. Probiotics in addition to metronidazole for treatment *Trichomonas vaginalis* in the presence of BV: a randomized, placebo-controlled, double-blind study. *Eur J Clin Microbiol Infect Dis.* 2020;39:345-351.
  145. Palma E, Recine N, Domenici L, Giorgini M, Pierangeli A. Long-term *Lactobacillus rhamnosus* BMX 54 application to restore a balanced vaginal ecosystem: a promising solution against HPV-infection. *BMC Infect Dis.* 2018;18(1):13. <https://doi.org/10.1186/s12879-017-2938-z>.
  146. Barthow C, Wickens K, Stanley T, et al. The Probiotics in Pregnancy Study (PiP Study): rationale and design of a double-blind randomised controlled trial to improve maternal health during pregnancy and prevent infant eczema and allergy. *BMC Pregnancy Childbirth.* 2016;16(1):133. <https://doi.org/10.1186/s12884-016-0923-y>.
  147. Wickens KL, Barthow CA, Murphy R, et al. Early pregnancy probiotic supplementation with *Lactobacillus rhamnosus* HN001 may reduce the prevalence of gestational diabetes mellitus: a randomised controlled trial. *Br J Nutr.* 2017;117(6):804-813. <https://doi.org/10.1017/S0007114517000289>.
  148. Slykerman RF, Hood F, Wickens K, et al. Effect of *Lactobacillus rhamnosus* HN001 in pregnancy on postpartum symptoms of depression and anxiety: a randomised double-blind placebo-controlled trial. *EBioMedicine.* 2017;24:159-165. <https://doi.org/10.1016/j.ebiom.2017.09.013>.
  149. Wickens K, Barthow C, Mitchell EA, et al. Maternal supplementation alone with *Lactobacillus rhamnosus* HN001 during pregnancy and breastfeeding does not reduce infant eczema. *Pediatr Allergy Immunol.* 2018;29(3):296-302. <https://doi.org/10.1111/pai.12874>.
  150. Martín V, Cárdenas N, Ocaña S, et al. Rectal and vaginal eradication of *Streptococcus agalactiae* (GBS) in pregnant women by using *Lactobacillus salivarius* cect 9145, a target-specific probiotic strain. *Nutrients.* 2019;11(4):810. <https://doi.org/10.3390/nu11040810>.
  151. De Alberti D, Russo R, Terruzzi F, et al. Lactobacilli vaginal colonisation after oral consumption of Respecta<sup>®</sup> complex: a randomised controlled pilot study. *Arch Gynecol Obstet.* 2015;292(4):861-867. <https://doi.org/10.1007/s00404-015-3711-4>.
  152. Russo R, Superti F, Karadja E, De Seta F. Randomised clinical trial in women with recurrent vulvovaginal candidiasis: efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses.* 2019;62(4):328-335. <https://doi.org/10.1111/myc.12883>.
  153. Ou YC, Fu HC, Tseng CW, Wu CH, Tsai CC, Lin H. The influence of probiotics on genital high-risk human papilloma virus clearance and quality of cervical smear: a randomized placebo-controlled trial. *BMC Womens Health.* 2019;19(1):103. <https://doi.org/10.1186/s12905-019-0798-y>.
  154. McMillan A, Rulisa S, Gloor GB, Macklaim JM, Sumarah M, Reid G. Pilot assessment of probiotics for pregnant women in Rwanda. *PLoS One.* 2018;13(6): <https://doi.org/10.1371/journal.pone.0195081>.
  155. Gille C, Böer B, Marschal M, et al. Effect of probiotics on vaginal health in pregnancy. EFFPRO, a randomized controlled trial. *Am J Obstet Gynecol.* 2016;215(5):608.e1-608.e7. <https://doi.org/10.1016/j.ajog.2016.06.021>.
  156. Husain S, Allotey J, Drymoussi Z, et al. Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. *BJOG: An Int J Obstet Gynaecol.* 2020;127(2):275-284. <https://doi.org/10.1111/1471-0528.15675>.
  157. Ho M, Chang YY, Chang WC, et al. Oral *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 to reduce group B *Streptococcus* colonization in pregnant women: a randomized controlled trial. *Taiwan J Obstet Gynecol.* 2016;55(4):515-518. <https://doi.org/10.1016/j.tjog.2016.06.003>.
  158. Olsen P, Williamson M, Traynor V, Georgiou C. The impact of oral probiotics on vaginal Group B Streptococcal colonisation rates in pregnant women: a pilot randomised control study. *Women Birth.* 2018;31(1):31-37. <https://doi.org/10.1016/j.wombi.2017.06.012>.
  159. Tomusiak A, Strus M, Heczko PB, et al. Efficacy and safety of a vaginal medicinal product containing three strains of probiotic bacteria: a multicenter, randomized, double-blind, and placebo-controlled trial. *Drug Des Dev Ther.* 2015;9:5345-5354. <https://doi.org/10.2147/DDDT.S89214>.
  160. Heczko PB, Tomusiak A, Adamski P, et al. Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: a randomised, double-blind, placebo-controlled trial. *BMC Womens Health.* 2015;15(1). <https://doi.org/10.1186/s12905-015-0246-6>.
  161. Marcotte H, Larsson PG, Andersen KK, et al. An exploratory pilot study evaluating the supplementation of standard antibiotic therapy with probiotic lactobacilli in South African women

- with bacterial vaginosis. *BMC Infect Dis.* 2019;19(1): <https://doi.org/10.1186/s12879-019-4425-1>.
162. Pendharkar S, Brandsborg E, Hammarström L, Marcotte H, Larsson PG. Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. *BMC Infect Dis.* 2015;15(1):255. <https://doi.org/10.1186/s12879-015-0971-3>.
  163. Daskalakis GJ, Karambelas AK. Vaginal probiotic administration in the management of preterm premature rupture of membranes. *Fetal Diagn Ther.* 2017;42(2):92-98. <https://doi.org/10.1159/000450995>.
  164. Kwak YK, Daroczy K, Colque P, Kühn I, Möllby R, Kopp KH. Persistence of lactobacilli in postmenopausal women—a double-blind, randomized, Pilot study. *Gynecol Obstet Invest.* 2017;82(2):144-150. <https://doi.org/10.1159/000446946>.
  165. Rostok M, Hütt P, Rööp T, et al. Potential vaginal probiotics: safety, tolerability and preliminary effectiveness. *Benef Microbes.* 2019;10(4):385-393. <https://doi.org/10.3920/BM2016.0123>.
  166. Mezzasalma V, Manfrini E, Ferri E, et al. Orally administered multispecies probiotic formulations to prevent uro-genital infections: a randomized placebo-controlled pilot study. *Arch Gynecol Obstet.* 2017;295(1):163-172. <https://doi.org/10.1007/s00404-016-4235-2>.
  167. Laue C, Papazova E, Liesegang A, et al. Effect of a yoghurt drink containing *Lactobacillus* strains on bacterial vaginosis in women—a double-blind, randomised, controlled clinical pilot trial. *Benef Microbes.* 2018;9(1):35-50. <https://doi.org/10.3920/BM2017.0018>.
  168. Anoshina TM. Role of microbiota correction in complex treatment of pregnant women with herpesvirus infection. *Perinatol I Pediatr.* 2016;4:22-25. <https://doi.org/10.15574/PP.2016.68.22>.
  169. Kovachev SM, Vatcheva-Dobrevska RS. Local probiotic therapy for vaginal *Candida albicans* infections. *Probiotics Antimicrob Proteins.* 2015;7(1):38-44. <https://doi.org/10.1007/s12602-014-9176-0>.
  170. Ribeiro AE, Monteiro NES, De MAVG, Costa-Paiva LH, Pedro AO. Can the use of probiotics in association with isoflavone improve the symptoms of genitourinary syndrome of menopause? Results from a randomized controlled trial. *Menopause.* 2019;26(6):643-652. <https://doi.org/10.1097/GME.0000000000001279>.
  171. MacPhee RA, Miller WL, Gloor GB, et al. Influence of the vaginal microbiota on toxic shock syndrome toxin 1 production by *Staphylococcus aureus*. *Appl Environ Microbiol.* 2013;79(6):1835-1842. <https://doi.org/10.1128/AEM.02908-12>.
  172. ELLEN® Probiotic Tampon. SCIENTIFIC SUMMARY product description. [https://www.ellentampon.ch/sites/all/themes/ellentampon\\_ch/media/downloads/probiotic-tampon-scientific-information-public.pdf](https://www.ellentampon.ch/sites/all/themes/ellentampon_ch/media/downloads/probiotic-tampon-scientific-information-public.pdf). Accessed November 2, 2020.
  173. Sauperl O, Zabret A, Fras ZL. Development of advanced sanitary materials with the use of probiotic paste. *J Eng Fiber Fabr.* 2020;15:155892502092221. <https://doi.org/10.1177/1558925020922215>.
  174. PRODUCTS. [www.fide.cz](http://www.fide.cz). <http://www.fide.cz/PRODUCTS.#unique-PRODUCTS>. Accessed November 2, 2020.
  175. Husmark UM, Gustafsson IA; Essity Hygiene and Health AB. Hygiene product with a probiotic composition. Pub.No.: US 2004/0243076 A1. December 2, 2004.
  176. Husmark UM, Gustafsson IA; Essity Hygiene and Health AB. Hygiene product with a probiotic composition. Pub.No.: DE 602004007788 T2. July 5, 2008.
  177. Zespół Ekspertów Polskiego Towarzystwa Ginekologicznego. Statement of the Polish Gynecological Society Expert Group on the use of ellen probiotic tampon. *Ginekol Pol.* 2012;83(8):633-638.
  178. Silva JA, De Gregorio PR, Rivero G, Abraham GA, Nader-Macias MEF. Immobilization of vaginal *Lactobacillus* in polymeric nanofibers for its incorporation in vaginal probiotic products. *Eur J Pharm Sci.* 2021;156:105563. <https://doi.org/10.1016/j.ejps.2020.105563>.
  179. De Gregorio PR, Silva JA, Rivero G, Abraham GA, Nader-Macias MEF; CERELA-CONICET-Universidad Nacional de Mar del Plata-Argentina. Method of immobilization of probiotic lactic acid bacteria textile substrate and product obtained. INPI N° 20180103893. December 27, 2018
  180. Cherpinski A, Szewczyk PK, Gruszczyński A, Stachewicz U, Lagaron JM. Oxygen-scavenging multilayered biopapers containing palladium nanoparticles obtained by the electrospinning coating technique. *Nanomaterials.* 2019;9(2): <https://doi.org/10.3390/nano9020262>.
  181. De Gregorio PR, Michavila G, Muller LR, et al. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. *PLoS One.* 2017;12(5):e0176930. <https://doi.org/10.1371/journal.pone.0176930>.
  182. Kersani D, Mougín J, Lopez M, et al. Stent coating by electrospinning with chitosan/poly-cyclodextrin based nanofibers loaded with simvastatin for restenosis prevention. *Eur J Pharm Biopharm.* 2020;150:156-167. <https://doi.org/10.1016/j.ejpb.2019.12.017>.
  183. Škrlec K, Zupančič Š, Prpar Mihevc S, Kocbek P, Kristl J, Berlec A. Development of electrospun nanofibers that enable high loading and long-term viability of probiotics. *Eur J Pharm Biopharm.* 2019;136:108-119. <https://doi.org/10.1016/j.ejpb.2019.01.013>.
  184. Nagy ZK, Wagner I, Suhajda Á, et al. Nanofibrous solid dosage form of living bacteria prepared by electrospinning. *Express Polym Lett.* 2014;8(5):352-361. <https://doi.org/10.3144/expresspolymlett.2014.39>.

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