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Review Article

Lactobacillus as probiotics: opportunities and challenges for potential benefits in female reproductive health

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Abstract: The growing interest of the scientific community in the study of probiotics has gathered valuable data about its beneficial effects for multiple clinical conditions. This data also provides evidence for the functions and properties of probiotics and how they contribute to health benefits by influencing normal microbiota. *Lactobacillus* is an important genus which has long been utilized in the food industry and is also found as normal oral, intestinal and vaginal microbiota. *Lactobacillus* has shown multiple health benefits but its relative importance as a probiotic is majorly explored for gastrointestinal health. Healthy vaginal microbiota typically harbors *Lactobacillus* spp. providing several health benefits for female reproductive health, but there is more data required in order to compare the relative benefits with probiotic *Lactobacillus* added through either natural food sources or with standard probiotics supplements. The present article discusses the current status of knowledge about vaginal *Lactobacillus* as a probiotic and also compares the potential of probiotics from natural sources and through supplements along with recent approaches in this area.

Keywords: *Lactobacillus*, probiotics, vaginitis, supplements, probiotic food

Introduction

As per the Food and Agriculture Organization and World Health Organization, probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. These organisms have several beneficial potentials giving them high recognition as human and animal nutritional supplements [2], such as antioxidant and anticancer activity, cholesterol reducing activity, anti-pathogenic, with beneficial microbiota management effects [2, 3], for all age groups including infants [4].

Ample studies have investigated diverse potential of probiotics in human health, however the majority of these are focused on gastrointestinal health. As gastrointestinal health affects whole body physiology, it is understood that these probiotics can exert their beneficial effects towards several other extra-gastrointestinal diseases [5].

The role of probiotics in vaginal health is an important domain highlighting their role in extra-intestinal benefits [6]. The vaginal microbiome is an important component regulating human vaginal health and *Lactobacillus* spp. are a major contributor to a healthy vaginal microflora [7]. *Lactobacillus* spp. play major roles in vaginal and reproductive health including prevention of pathogens through production of antimicrobial compounds and metabolites [8]. Notwithstanding, the vaginal microbiota is affected by several factors including, sexual practices, menstruation, pregnancy, antibiotics application, and vaginal douching thereby affecting vaginal health [9]. The depletion of *Lactobacillus* spp. in vaginal microflora along with overgrowth of non-*Lactobacillus* bacteria often linked with several diseases, such as bacterial vaginosis and sexually transmitted infections [6, 10].

Due to the important role of *Lactobacillus* in vaginal and female reproductive health, some-

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times these bacteria may need to be supplemented in order to correct vaginal microbiota dysbiosis. However, the establishment of supplemented strains over other strains provided through natural sources is comparatively different. In addition, recent studies are trying to understand beneficial effects by natural vs supplemented *Lactobacillus* strains for vaginal health. The present article tries to gather information about the application of *Lactobacillus* as probiotics for vaginal health and also covers recent approaches to understand the effects of these probiotic strains on human health including effects on vaginal and female reproductive health.

Vaginal microbiota and the role of *Lactobacillus*

Vaginal microbiota display peculiar colonization pattern for each female, however the *Lactobacillus* genera make a major contributor for healthy vaginal microbiota [11]. Studies have linked the presence of *Lactobacillus* in the endometrial microbiome with early pregnancy, implantation failure or early spontaneous abortion [12]. Despite technological advancements and growing interest of the scientific community in understanding vaginal microbiota, healthy composition is not completely understood due to its complex community structure and dynamics despite its critical role in female reproductive health [13]. Vaginal Lactobacilli are an important microbiota component playing multiple roles, including vaginal innate immunity, production of lactic acid leading to low vaginal pH (between 4.0-4.5) inhibiting several pathogens, productions of bacteriocins and checking vaginal inflammation. The vaginal microbiota is subjected to changes with different stages of life i.e. infant, puberty, pregnancy and menopause [14]. In addition, temporal variations in vaginal microbiota are attributed to hormonal changes, antibiotics use, menstruation and vaginal douching [15, 16].

Vaginal microbiota shows different composition among ethnic groups and is defined as community state type (CST). The type of vaginal microbiota is clustered into five CSTs referred as I, II, III, IV, V; on the basis of the relative abundance of four *Lactobacillus* spp. i.e. *L. iners, L. gasseri, L. crispatus,* and *L. jensenii* along with heterogenous group of microbes. *L. crispatus* dominates CST I, *L. gasseri* by CST II, *L. iners* and *L. jensenii* by CST III and V respectively, while CST IV is considered as dysbiotic state where multiple obligate and facultative anaerobes (such as *Bifidobacterium, Gardnerella, Prevotella, Aerococcus, Atopobium, Anaerococcus, Dialister, Eggerthella, Finegoldia, Gemella, Parvimonas, Peptoniphilus, Peptostreptococcus, Megasphaera, Sneathia, Veillonella*, and *Corynebacterium* with some rare taxa) dominates the microbiome with deficiency of *Lactobacillus* [17, 18]. Some additional CST types have recently been identified after classification of existing CST type IV into subtypes as CST IV-A, CST IV-B, and CST IV-C along with CST VI and CST VII containing dominant *Gardnerella vaginalis* and *Prevotella,* respectively, type VIII resemble aerobic vaginitis, and type IX predominantly having genera other than *Lactobacillus*, *Gardnella* or *Prevotella* [18]. Some studies related to *Lactobacillus* spp. *that have been* found in healthy vaginal microbiota are represented in **Table 1** in order to provide overview about different vaginal *Lactobacillus* strains.

Due to highly complex and diverse composition of vaginal microbiota and the important roles played by *Lactobacillus* in normal physiology and status, it is important to understand the influence of *Lactobacillus* on the function in vaginal microbiota. **Table 2** lists some of the studies indicating roles of different vaginal microbiota *Lactobacillus* spp. under different conditions. These studies indicate that *Lactobacillus* plays a major role in shaping *the* structure of vaginal microbiota as indicated by different CSTs. These CSTs indicate towards specific microbiota-host interactions and their impact on host health which deserve critical appraisal for understanding probiotic potential of *Lactobacillus*. In addition to the role of CST in vaginal microbiota-host interactions, host physiology also greatly affects *Lactobacillus* species in vaginal microbiota. The complexity of vaginal microbiota as evident through CSTs indicates the requirement of advanced tools and approaches to understand exact effect of vaginal microbiota on human health. This will also help to harness *the* true potential of this knowledge and therefore *in the* next section *we will* try to give *a* brief overview about recent approaches in this area.

Table 1. Some representative studies specific to *Lactobacillus* strain found in healthy vaginal microbiota

Lactobacillus strains observed in healthy vaginal microbiota	Age of participants	Reference
L. reuteri (32.5%), *L. fermentum* (25%), and *L. salivarius* (16.25%)	18-45 yrs	[19]
L. crispatus (39.6%), *L. gasseri* (45.8%), and *L. jensenii* (14.6%)	18-45 yrs	[20]
L. mucosae (16.2%), *L. fermentum* (6.4%), *L. gasseri* (3.9%), *L. brevis* (3.2%), *L helveticus (2%), L. jensenii* (2%), *L. ingluviei* (1.3%), *L. reuteri* (1.3%), *L. gallinarum* (0.6%) and *L. parafarraginis* (0.6%)	18-35 yrs	[21]
L. crispatus (26.2%), *L. gasseri* (31%), *L. jensenii* (11.9%), *L. vaginalis* (9.5%), *L. mucosae* (2.4%), *L. reuteri* (2.4%), *L. fermentum* (2.4%), *L. oris* (2.4%) and *L. delbrueckii* (2.4%) (Found in HIV negative subjects during the study on HIV positive and negative women)	33-39 yrs	[22, 23]
L. iners, L. crispatus, L. gasseri, and L. *jensenii*	18-40 yrs	[24]
L. helveticus, *L. iners* and *L. gasseri*	19-35 yrs	[25]
57 Lactobacillus strains were purified including *L. crispatus*, *L. jensenii*, *L. gasseri*, *L. mucosae*, and *L. vaginalis*	16-22 yrs	[26]
L. crispatus, *L. jensenii*, and *L. iners* (were detected to check effects of vaginal washing on *Lactobacillus*)	---	[27]

Table 2. Role of Vaginal *Lactobacillus* in different clinical conditions and maintenance of normal physiological response

Lactobacillus spp.	Activity	Reference
L. crispatus, L. jensenii, L. gasseri	Cell free supernatant of *Lactobacillus* spp. showed inhibition of proliferation of cervical cancer using Caski cell lines and showed pH independent morphological changes. Human papillomavirus (HPV) E6 and E7 oncogenes, along with CDK2 and cyclin A were reduced, while p21 expression was increased. This inhibitory effects on cervical cancer cells are mediated by HPV oncogenes and cell cycle-related genes regulation.	[28]
L. crispatus	*L. crispatus* inhibited entry of Herpes simplex virus (HSV)-2 in the cell by trapping virus particles on cell lines based assays.	[29]
L. acidophilus, *L. gasseri*, *L. jensenii*	*Lactobacillus* spp. isolated from vaginas of healthy women self aggregated, adhered to vaginal epithelia and displaced vaginal pathogens, such as *G. vaginalis*. The self aggregation was mediated through proteins in *L. gasseri* and lipoproteins in *L. acidophilus* and *L. jensenii*. The competition for vaginal cells glycolipid receptors between *Lactobacillus* and pathogenic bacteria was proposed as a target for this activity.	[30]
L. crispatus	*Lactobacillus* was co-cultured with vaginal epithelial cell lines (MS74) and evaluated for their activity on vaginal epithelial healing using scratch assay. The bacteria significantly improved re-epithelialization of MS74 cells with increased vascular endothelial growth factor (VEGF).	[31]
L. iners, L. crispatus	3D human vaginal epithelial cells were colonized with studied *Lactobacillus or Atopobium vaginae* and *Prevotella bivia* (associated with bacterial vaginosis) and different group of bacteria act differentially in modulating innate immune response and barrier function.	[32]
L. crispatus, L. iners, L. gasseri, L. jensenii	Level of extracellular matrix metalloproteinase inducer and matrix metalloproteinase (MMP-8) was evaluated in vaginal secretions and compared with vaginal bacterial communities and found that vaginal microbial communities affect these parameters.	[33]
L. crispatus, L. iners	Dominance of studied *L. crispatus* in vaginal microenvironment of pregnant women shows lower induction of stress related proteins HSP70.	[34]
L. crispatus, L. iners	The relative concentrations of *L. crispatus*, *L. iners* and *G. vaginalis* decide the vaginal microbiota composition of mid-trimester pregnant women.	[35]

Probiotic *Lactobacillus*: natural and supplemented strains

Several bacterial genera are used in probiotic preparations, including; *Lactobacillus*, *Bifidobacterium, Enterococcus, Escherichia, Streptococcus* and *Bacillus*. Along with this, certain fungal strains such as *Sacchromyces* are also used for probiotic application [36]. Though probiotic bacteria are generally a component of healthy microbiota and confers normal health status, their reduction/absence under disease conditions needs their administration into dysbiotic microbiota in order to provide health benefits. However, the efficacy of probiotics depends on species, dose, clinical disease and duration of application [37] and therefore sometimes it is required to add certain specific probiotic strains to provide health benefits. There are two major ways to incorporate probiotics for health benefits. The probiotics naturally present in certain food products or certain medicinal formulations can be used as probiotic supplements in order to provide benefits in dysbiotic microbiota. *Lactobacillus* spp. are naturally present in several fermented food products. Such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* which are present in yoghurt [38]. Sauerkraut is a product of cabbage fermented by lactic acid bacteria and contains a large proportion of *Lactobacillus* spp. [39]. Kimchi is a popular fermented food of Korea containing high level of lactic acid bacteria such as, *Lactobacillus plantarum* [40]. Soymilk, Kefir and several other food products provides *Lactobacillus* as natural probiotic bacteria [41, 42]. In addition, several *Lactobacillus* supplements are also available in markets for health benefits.

The choice of probiotics from natural sources vs. probiotics supplements depends on a number of factors. The probiotic food can offer multiple beneficial bacteria and thus may provide broader health benefits over supplemented strains having a specific type of bacteria. In addition, probiotics taken from food may allow greater chance to interact with digestive tract naturally as they need to compete with existing microbiota for nutrients and adhesion - due to colonization resistance [43], and therefore food probiotics are considered to provide better optimization for colonization and subsequent beneficial effects. In contrast, the efficacy of probiotics in food will depend on several factors, such as processing, storage and individual variations in food components. Therefore, probiotic supplements can provide standardized and consistent doses of specific strains in order to ensure reliable and predictable outcomes.

The recent concept of synbiotics integrates probiotics with the nutritional requirements for their growth and survival. These nonviable food components confer health benefits to the host due to promoting healthy microbiota is termed as prebiotics and the combination of synergistically acting probiotics-prebiotics is known as synbiotics [44]. Each type of probiotic formulation has their unique advantage and disadvantages and is a topic of discussion among researchers.

Recent approaches for evaluation of probiotic potential of *Lactobacillus*

Considering the important role of vaginal *Lactobacillus* in maintaining healthy status, it is important to evaluate their probiotic potential for particular clinical conditions in order to develop and identify an effective probiotic strain. **Figure 1** represents the role of *Lactobacillus* as probiotics along with their sources. It is proposed that the vaginal ecosystem embodies several probiotic strains which can be used for the development of functional probiotic formulations [45].

Microorganisms should qualify certain criteria to be a useful probiotic along with their demonstrated beneficial effects. These include their non-pathogenic and non-toxic nature, free from any side effects, able to survive in the body, present in sufficient numbers to provide health benefits, and compatibility with formulation components, storage and processing conditions [46]. The evaluation of each aspect requires separate set of assays to understand the probiotic potential of any microorganism. Evaluation of safety with probiotic strains is highly important after their cultivation. Assessment of safety profile involves several tests, such as evaluation of infectivity and *in situ* toxin production, presence of ability to transfer antibiotic resistance genes, any potential harmful metabolic activity along with immunological effects [47]. These assessments are important to ensure safety profile of a probiot-

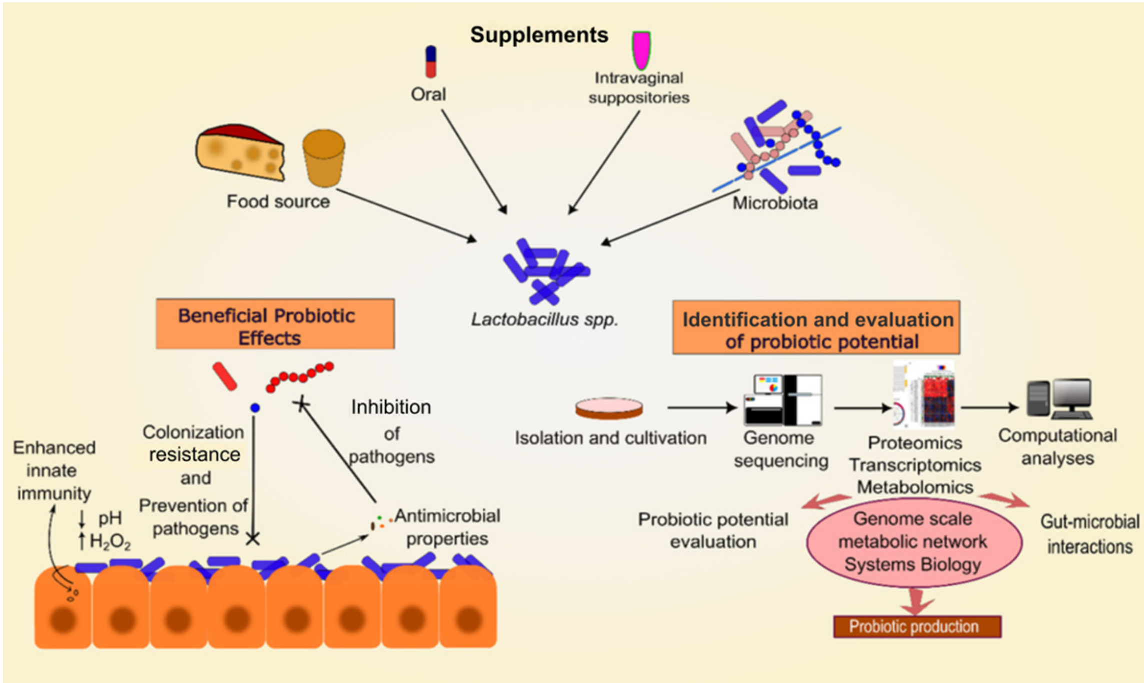

Figure 1. Probiotic potential of *Lactobacillus* for female reproductive health and approaches for identification and evaluation of their beneficial potential.

ics. For instance, a recent study has evaluated haemolytic activity, bile salt hydrolase activity, and antibiotic susceptibility to assess safety profile of *Lactobacillus* probiotics. In addition, the ability to resist low pH, bile and lysozyme, antagonistic property for pathogens, ability to auto/co-aggregate, production of hydrogen peroxide, biofilm formation, and adhesion to vaginal epithelial cells were considered for probiotic functional property of *Lactobacillus* [45].

Recent technological advancements enabled a deeper understanding of the mechanisms, effects, and applications of probiotics. The use of omics technologies, advancement in formulation development with innovative research approaches has promised the way for significant progress in probiotic study. The integration of multi-omics, high throughput approaches with animal and human studies are providing additional insights about effects of probiotics [48]. Systems biology is also providing valuable information about basic biology and the probiotic potential of vaginal *Lactobacillus* [49]. Therefore, the development of multi-omics technologies along with systems biology approaches and integration of biological assays for evaluation of functional and safety profile of probiotics is paving the way for identification of efficient probiotic strains for multiple applications including vaginal probiotics.

Challenges associated with the development of *Lactobacillus* as probiotics

Despite several technological advancements and improved understanding of probiotic potential of *Lactobacillus*, their development as an effective agent faces several challenges. The efficacy of probiotic *Lactobacillus* is either strain/or disease specific. For instance, the anti-*Candida* effects of vaginal Lactobacilli is evident, however, some *Lactobacillus* strains such as *L. crispatus* BC2, *L. gasseri* BC10 and *L. gasseri* BC11 act as most active strains for this activity. In addition, this activity is not found against all *Candida* strains, such as *C. krusei* and *C. parapsilosis* [50]. Moreover, age, probiotic strain, species, duration and dosage of intervention along with the form of administration also affect the efficacy of probiotics [51]. Along with probiotic and host associated efficacy variations, colonization of supplemented probiotic strains faces several challenges due to the complex structure of gut and vaginal

Table 3. Different techniques and methods used for preparation of probiotic *Lactobacillus* formulations

Lactobacillus Formulation	Method	Reference
Lactobacillus acidophilus	Encapsulation of *L. acidophilus* alginate (Al) and alginate-chitosan (Al/Chi) through extrusion method to improve survival in intestine and freeze drying.	[58]
Lactobacillus plantarum 299v	Lyophilization has benefit to probiotics from harsh conditions. Microencapsulation was done to protect the probiotics by coating them.	[59]
Lactobacillus reuteri	Coacervation was used for probiotic microcapsules with benefits in terms of protective capabilities and viability.	[60]
Lacticaseibacillus rhamnosus GG (Lactobacillus rhamnosus)	Encapsulation of *L. rhamnosus* GG strain in alginate systems was performed through ionotropic gelation technology. It is a method in which polyelectrolytes react with oppositely charged molecules which are subjected to sol-gel transition resulting in the formation of structured physical materials.	[61]
Lactobacillus acidophilus LA-5	Bacteria were microencapsulated to reduce the inhibition, and increased viability of the microencapsulated cultures. Bacterial damage during the microencapsulation by the emulsion method was indicated as low.	[62]
Lactobacillus sporogenes	Microencapsulation of probiotics using alginate beads has proven to improve viability of probiotic bacteria in gastric conditions.	[63]
Lactobacillus brevis	*L. brevis* loaded in buccal films by means of a modified casting-solvent evaporation method was able to deliver Lactobacilli inside the buccal cavity. This process involves the evaporation of a solvent from a starting solution and the subsequent formation of a polymeric membrane.	[64]

microbiota. A probiotic organism receives the first challenge from existing microbial population, as they compete for nutrients and colonization sites and therefore, colonization of any probiotic strain defines its ability to influence its efficacy. The colonization of probiotics is influenced by several factors, such as local microbiota composition, ability of supplemented strain to withstand effects of acid, bile salts, enzymes, host immunity etc. [52-54]. In addition, genetic determinants of probiotic stains including specific genes and mechanisms involved in adhesion, biofilm formation, and mucosal interaction which also contribute to the successful colonization of probiotic strains [55].

Several approaches have been tried to improve the probiotic potential of supplemented strains. For example, microencapsulation leads to valuable effects on increasing cell viability during storage and needs investigation to understand their impact on probiotics health benefits [56]. **Table 3** presents approaches utilized for production of commercial formulations of probiotics. Considering the status of supplemented strains in colonizing the host and showing health benefits, the probiotics taken with fermented food have shown their ability to transiently alter intestinal microbiota composition. The use of certain species in fermented food may increase the colonization. It is noteworthy that, some probiotic foods are available only in some countries therefore, their use and efficacy may differ as per the location, food habits, living style and environment etc. [57].

The route of administration of probiotics for benefits in vaginal health is also a challenge. Recent clinical trials demonstrated that vaginal colonization of Lactobacilli is low after oral administration of *L. rhamnosus GR-1* and *L. reuteri RC-14* [65]. In addition, microbiota composition and physical conditions significantly influence probiotic colonization and persistence of supplemented strains [44]. For instance, sexually active women show less chances of colonization of supplemented probiotic strains [53]. Moreover, long term use of antibiotics, hormonal changes, glycogen content, and vaginal pH can also influence colonization and efficacy of supplemented probiotic strains [66]. These challenges need careful consideration before designing probiotics for female reproductive health applications.

Potential of probiotics from natural sources and supplements

Lactobacillus strains supplemented through natural sources has gained significant attention due to their diverse health promoting properties. However, using *Lactobacillus* from natural sources vs. supplements present both challenges and opportunities. Meeting the growing demand of probiotics from natural sources requires proper availability of prebiotics, cost effective production of probiotics, and maintenance of viability and storage of functional food containing probiotics during manufacturing and supply [67]. In addition to production, the commercialization, regulatory approval and guidelines to use natural *Lactobacillus* strains as probiotics require rigorous scientific and regulatory support [68]. Therefore, probiotic manufacturers need to ensure proper efforts with regulatory bodies to ensure safety, efficacy, and quality control of natural *Lactobacillus*-based probiotic products. The use of *Lactobacillus* as supplement in vaginal suppositories is also investigated for recurrent urinary tract infection with positive effects; however, more focused study on *Lactobacillus* strain, dosing is required to assess the actual potential [69].

These regulatory considerations also involve safety of natural *Lactobacillus* as there are some studies that have reported *Lactobacillus*-associated infections. Bacteremia due to *Lactobacillus* is rare but is found in immunocompromised patients, and it must be evaluated for avoiding potential risk. Studies reported that *L. rhamnosus* GG, *L. plantarum*, and *L. paracasei* were involved in bacteremia with certain other bacteria [70], especially among immunocompromised patients. In addition, probiotic organisms are also known for causing systemic infections, metabolic disturbances, and may induce the immune system or contribute to horizontal gene transfer leading several adverse effects [71]. Though, *Lactobacillus* strains hold promise to show therapeutic potential in various diseases, their safety evaluation needs careful consideration and possess an important challenge.

Conclusion

It can be concluded that probiotic *Lactobacillus* strains obtained from natural sources as well as supplements holds both challenges and possibilities. The recent advancement in this field with the addition of high throughput approaches has greatly improved our understanding of *Lactobacillus* mediated beneficial effects and their further development as therapeutics. The probiotics from natural sources as well as from supplemented strains shows potential in many aspects. Overcoming production challenges, ensuring safety, exploring targeted applications, and advancing personalized and combination therapies are key areas of focus. Continued research, innovation, and evidence-based practice will pave the way for harnessing the full potential of natural *Lactobacillus* as probiotics.

Disclosure of conflict of interest

None.

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