Vaginal Microbiota

L. crispatus LbV 88

L. jensenii LbV 116

L. gasseri LbV 150N

L. rhamnosus LbV 96

Anthony P. Thomas, PhD

Rationale and Clinical Research of Vaginally-Derived Probiotic Strains to Support Female Urogenital Tract Health

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Rationale and Clinical Research of Vaginally-Derived Probiotic Strains to Support Female Urogenital Tract Health

This booklet reviews current understanding of the vaginal microbiota and its relationship to women's health, as well as the rationale and clinical research to demonstrate the therapeutic potential of vaginally-derived probiotic strains L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 to support vaginal microbial ecosystem balance. References to original research are provided for more in-depth review by medical and health-care professionals.

The failure of most medical education programs to adequately teach future physicians about the human microbiota, its relationship to health, and appropriate clinical applications of specific, validated probiotic strains and multi-strain formulations for a given health condition, ultimately diminishes care for patients. It is critical that healthcare practitioners acknowledge the human microbiota and consider its role in health maintenance. Clinical research, dissemination of research results, and education will be key, as confusion about what constitutes a true probiotic-based intervention and misinformation are widespread.

Anthony P. Thomas, PhD

Introduction

Complex microbial communities (microbiota), including their collective genetic material (microbiome), differ between anatomic sites of an individual (e.g., intestinal, vaginal, oral, skin) as well as between people [1]. An increasing body of scientific evidence has demonstrated these microbial communities markedly influence human health. Clinical research has shown the targeted manipulation of these microbial communities with specific probiotic strains offers a promising strategy to improve and maintain health.

The composition and function of the vaginal microbiota has been linked to women's health. Microorganisms (microbes) inhabiting the vagina provide the first line of defense in the urogenital tract. Although there is not a definitive "normal" vaginal microbiota, current scientific knowledge has revealed that Lactobacilli predominance is generally the hallmark of a healthy vaginal microbiota.

High vaginal Lactobacilli abundance is associated with the promotion and maintenance of vaginal microbial ecosystem balance. Low numbers of vaginal Lactobacilli or absence is more often associated with increased risk of bacterial vaginosis (BV), yeast vaginitis ("yeast infection"), aerobic vaginitis (AV), urinary tract infections (UTIs), and adverse obstetric outcomes (e.g., miscarriage, premature rupture of membranes (PROM), preterm birth) [2-6].

The application of probiotics to support women's vaginal health in clinical practice has gained increasing recognition as both a primary and adjuvant therapy, as well as for prophylactic prevention of vaginal microbial ecosystem imbalance. It is worth noting the current internationally endorsed definition of probiotics established by an expert panel commissioned in 2001 by the Food and Agriculture Organization (FAO) of the United Nations and supported by the World Health Organization (WHO), which states, "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." The majority of fermented foods and products labeled as containing probiotics on the market have not been appropriately tested and verified as such. How "probiotic" containing products are regulated, marketed, and sold often has nothing to do with the definition.

It is well recognized scientifically that probiotics are strain, dose, and condition specific. Strain functionality and associated health claims beyond general support of gastrointestinal health in humans require substantiation of efficacy with clinical trials. Thus, specific probiotic strain(s) indicated to support women's urogenital tract health must be provided in sufficient quantity as validated by clinical research. Otherwise, it does not constitute a true probiotic for this condition.

Most probiotic products marketed for support of women's vaginal health are not supported by clinical research for this indication. In contrast, the vaginally-derived strains L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96, representing the predominant vaginal Lactobacillus species of healthy women, are clinically validated to promote vaginal microbial ecosystem balance in support of urogenital tract health with daily oral supplementation.

These patented, unique strains have been scientifically characterized and selected among many candidate strains of these four Lactobacillus species. Research demonstrated these strains are safe, capable of surviving during gastrointestinal transit (requisite for oral use), and display desired probiotic attributes to support women's urogenital tract health. Multiple clinical studies have shown that oral supplementation with these strains helps restore and maintain vaginal Lactobacilli, and acidifies the vaginal to antagonize growth of vaginal pathogens.

Protective Vaginal *Lactobacilli*

Bacterial species of the genus Lactobacillus are the predominant microbes inhabiting the vaginal tract in the majority of reproductive age women. Lactobacilli play a major role in protecting the vaginal environment against infection by pathogenic microbes. Displacement of Lactobacilli from the vagina can upset the microbial balance to compromise health.

Lactic Acid Production & Vaginal Acidification

Lactobacilli produce lactic acid from the fermentation of glucose [7]. Vaginal Lactobacilli are the primary source of lactic acid in the vagina [8, 9] and only source of the D-isomer as human cells only produce L-lactic acid. Within a Lactobacilli-dominated vaginal microbiota, the production of lactic acid and vaginal acidification plays a prominent role in imparting the broad protection against other undesirable microbes [10]. Maintenance of an acidic vaginal pH reinforces Lactobacilli predominance to support a balanced vaginal microbiota with limited microbial diversity.

In addition to vaginal acidification, lactic acid has been shown to directly inactivate various reproductive and urinary tract pathogens [11-13].

The production of the D-lactic acid isomer by some Lactobacillus strains enhances protection against microbial invasion of the upper genital tract by supporting the integrity of the cervical external orifice of the uterus [14]. The majority of preterm births result from infections caused by bacteria from the vagina, thus requires they traverse the cervix.

Protective Mechanisms

In addition to lactic acid production and vaginal acidification, Lactobacilli are thought to utilize several mechanisms to inhibit pathogen colonization of the vagina [15]:

- co-aggregation of undesirable microbes to prevent colonization
- production of biosurfactants to disrupt adhesion to the vaginal mucosa and biofilm disruption
- production of antimicrobial bacteriocins and hydrogen peroxide (H_2O_2)
- competitive exclusion of other microbes via competing for nutrients and host surfaces
- reinforcing the integrity of the vaginal mucosal epithelial barrier
- regulation of host immune responses (production of antimicrobial peptides/proteins such as defensins, lactoferrin and lysozyme, and alkaline phosphatases, which can bind to lipopolysaccharide/endotoxin to neutralize toxicity)

Vaginal Microbiota

The vagina is a dynamic environment colonized by various microbes. Microbial species that inhabit the vaginal tract play an important role in the maintenance of health and prevention of infection. Imbalances in the proportion of microbes (dysbiosis) can increase risk of infection and reproductive complications.

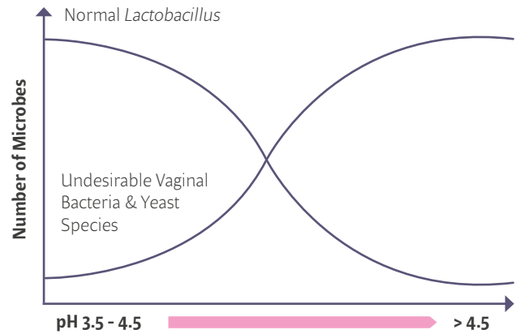

Fig 1. Relationship between vaginal *Lactobacilli* abundance and acidity (pH) of vagina.

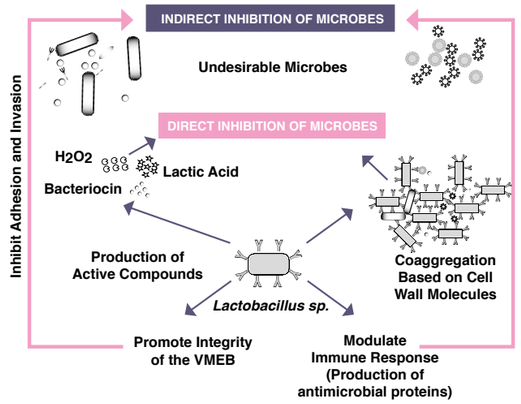

Fig 2. Potential mechanisms by which vaginal *Lactobacilli* support vaginal microbial ecosystem balance and urogenital tract health. Adapted from J. Younes *et al. Trends in Microbiology* 2018

Composition

Unlike any other anatomical site of the human body, most vaginal microbial communities are dominated (>50%) by one or more *Lactobacillus* species. Recent studies have shown that composition and relative abundance of vaginal microbial communities in reproductive-aged women cluster into at least 5 core vaginal microbiota, termed community state types (CSTs) [16-18]. Each of four of these CSTs, representing the majority of women (>70%), are dominated by a different *Lactobacillus* species: *L. crispatus* (CST I), *L. gasseri* (CST II), *L. jensenii* (CST V), and *L. iners* (CST III). CST IV is characterized by low proportions or absence of *Lactobacilli*, and is composed of a diverse mixture of primarily anaerobic bacteria including species of the genera *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella* and other taxa in the order *Clostridiales*, as seen with states of bacterial vaginosis (BV). Although CST IV can be observed in otherwise healthy women, asymptomatic for BV, it is associated with higher Nugent scores and is a risk factor for adverse gynecologic and obstetric outcomes [3, 19, 20]. Nugent scores are determined using a Gram stain scoring system from 0 – 10 for vaginal swabs reflecting the relative abundance of Gram-positive rods (*Lactobacilli*) and Gram-negative variable rods and cocci (*G. vaginalis, Prevotell*a, etc.) to diagnose BV, with 0 – 3 considered normal, 4 – 6 indicative of intermediate bacterial counts, and 7 – 10 diagnostic of BV.

Vaginal microbiotas dominated by *L. iners* have lower vaginal concentrations of D-lactic acid as *L. iners* cannot produce this lactic acid isomer [14], which may in part mediate the higher observed frequency of BV and preterm delivery in these women [21]. *L. iners* also does not produce H2O2. In contrast, *L. crispatus*, *L. gasseri*, and *L. jensenii* are all producers of both D-lactic acid and H2O2. H2O2-producing *Lactobacilli* are more likely to sustain long-term vaginal colonization, and women colonized by H2O2-producing *Lactobacilli* have decreased acquisition of human immunodeficiency virus (HIV) infection [22], gonorrhea [22], and BV [23]. Evidence supports a vaginal microbiota dominated by *Lactobacillus* species other than *L. iners* is optimal to support vaginal health [17, 18].

Ethnic Differences

Differences in the composition of the vaginal microbiota has been observed between women of different ethnic backgrounds. Lactobacilli-dominated vaginal microbial communities (CST I, II, III, V) were observed in 80-90% of Asian and white women, but only 60-62% of Hispanic and black women [16]. Such differences in vaginal microbial communities between women of different ethnic backgrounds has been observed in other studies as well [18, 24]. Overrepresentation of CST IV in Hispanic and black women has been associated with a higher vaginal pH in these ethnic groups.

I	*L. crispatus*	Healthy State
II	*L. gasseri*	Healthy State
V	*L. jensensii*	Healthy State
III	*L. iners*	Healthy or Transitional?
IV	Diverse Anaerobes	Dysbiosis / BV

Table 1. Core vaginal bacterial community state types (CSTs) dominated by a *Lactobacillus* species or low/absent vaginal *Lactobacilli* (IV). Adapted from J. Ravel *et al. PNAS* 2011

ETHNICITY	MEDIAN pH VALUES	% OF COMMUNITIES DOMINATED BY Lactobacillus SPECIES
HISPANIC	5.0	59.6
BLACK	4.7	61.9
ASIAN	4.4	80.2
WHITE	4.2	89.7

Table 2. Differences in Lactobacilli predominance of vaginal microbiota and vaginal pH of ethnic groups. Adapted from J. Ravel et al. PNAS 2011

Pregnancy & Estrogen

Composition of the vaginal microbiota can be dynamic and capable of rapid shifts within a short period of time (e.g., < 24 hours). Vaginal microbial communities of pregnant women are more stable and have higher relative abundance of Lactobacilli [25, 26]. The most common protective vaginal Lactobacillus species observed in healthy pregnant women were L. crispatus and L. gasseri (>50%), followed by L. jensenii (~20%) and L. rhamnosus (~10%), as well as combinations thereof (~10%) [27].

Estrogen plays an important role in promoting a Lactobacilli-dominated vaginal microbiota [28, 29]. Nutrient containing vaginal secretions and glycogen-containing vaginal epithelial cells, that are sloughed and subsequently lysed, are thought to be primary nutrient sources for the vaginal microbiota.

Estrogen increases the volume of vaginal secretions and induces thickening of the vaginal epithelium along with glycogen accumulation, thought to support growth of glucose-fermenting Lactobacilli [30]. Changes in relative abundance of vaginal Lactobacilli are associated with both estrogen levels and glycogen content across the various life-stages of women (e.g., pre-pubertal, pubertal/reproductive age, postmenopausal) [31].

Loss of Vaginal Lactobacilli: Common Factors

Various common factors influence the vaginal microbiota and have been associated with dysbiosis/bacterial vaginosis (BV): hygiene/intravaginal practices (douching), sexual activity (e.g., increased frequency and number of partners, lack of male circumcision/condom use), stress, smoking, and use of anti-biotics/-fungals [3, 32].

Antimicrobials have been the primary therapeutic intervention utilized for the treatment of urogenital infections for more than 4 decades. Unfortunately, antimicrobial treatment is often ineffective, particularly for BV, and there is a high rate of recurrence. Efficacy is also diminishing with increasing development of antimicrobial resistance. The antimicrobial, metronidazole, is the most commonly used treatment for BV; however, cure rates associated with this treatment are low (as low as 61% one month post therapy [33]) with a high incidence of overgrowth of undesirable bacteria following treatment [34].

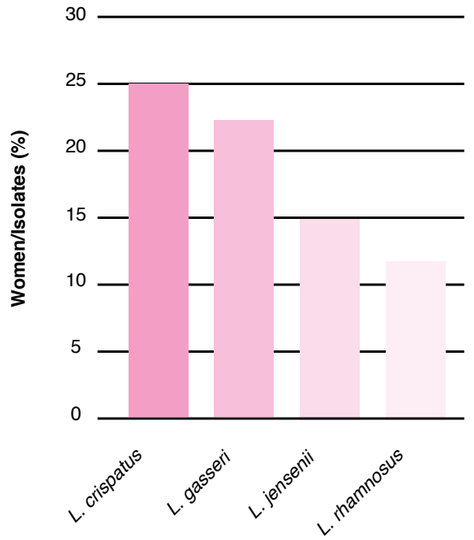

Fig 3. Frequency of detected Lactobacillus species in healthy pregnant women. Adapted from H. Kiss et al. BJOG 2007

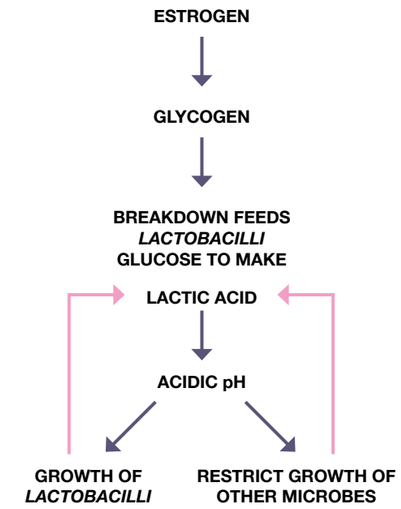

Fig 4. Positive influence of estrogen on vaginal lactic acid production and reinforcement of vaginal Lactobacilli abundance. Adapted from J. Haya *et al. Open J Obstetrics and Gynecology* 2014

Loss of Vaginal Lactobacilli: Antibiotics

Antibiotics affect not only pathogenic microorganisms, but many human residential symbiotic and administered probiotic bacterial strains too. Many strains of the most prevalent vaginal Lactobacilli species (i.e., L. crispatus, L. iners, L. jensenii, and L. gasseri) were all demonstrated to be susceptible to commonly used systemic antibiotics including ampicillin, cefazolin, cefotaxime, and vancomycin [35].

Loss of Vaginal Lactobacilli: Bacterial Vaginosis & Urinary Tract Infections

Many women experience a transient, often recurrent, loss of a Lactobacilli-dominated vaginal microbiota and reduced vaginal acidity, both of which are associated with increased risk of urogenital infections, as the reduction in Lactobacilli makes for a more conducive vaginal environment for the proliferation of many anaerobic bacteria such as Gardnerella vaginalis (G. vaginalis) and Atopobium vaginae (A. vaginae).

BV and UTIs are common infections, afflicting hundreds of millions of women annually [36, 37], with BV as the most common cause of vaginal symptoms among women. BV is a risk factor for acquisition of both bacterial (e.g., gonorrhea, chlamydia) and viral (HIV, HSV, and HPV) sexually transmitted diseases, Trichomonas vaginalis infection, as well as adverse obstetric outcomes (e.g., miscarriage, fetal distress syndrome, PROM, preterm birth) [3, 6, 38].

G. vaginalis and A. vaginae are commonly associated with BV [39, 40], whereas the majority (>80%) of UTIs are caused by uropathogenic E. coli (UPEC) and often associated with aerobic vaginitis (AV) [41]. These bacteria colonize the vagina via the formation of biofilms, which results in increased tolerance to adverse conditions for better persistence in hostile environments (i.e., protection from the immune system and decreased susceptibility to antibiotics) [42, 43].

Adherent biofilm comprised of mostly G. vaginalis and A. vaginae was observed to persist for 3 weeks following 1-week treatment with orally administered metronidazole in women with BV [40]. In the UK, BV is frequently treated with topical clindamycin. The proportion of group B. streptococci isolated from neonatal blood cultures that are resistant to clindamycin or erythromycin has risen substantially over recent years in the UK (Health Protection Report 2013, 7:46), most likely as a result of exposure to these antibiotics.

Preterm Birth & Premature Rupture of Membranes: The Importance of Lactobacillus crispatus Within The Vaginal Microbiota

Preterm birth is the leading cause of infant morbidity and mortality globally. An estimated 15 million babies are born preterm annually, and this number is increasing, with annual healthcare costs for surviving babies in excess of $25 billion [44-48]. The most frequent cause of preterm birth is ascending bacterial infection from the vagina through the cervix and into the uterine cavity [49].
The vaginal microbiota during pregnancy is generally characterized by higher relatively abundance of Lactobacilli and community stability with decreased microbial diversity. Women who are deficient in vaginal Lactobacillus species are at increased risk of vaginal microbial ecosystem imbalance, characterized by an elevated vaginal pH from reduced lactic acid concentrations, which can lead to overgrowth of diverse, undesirable microbes less protective of urogential tract health. BV and loss of vaginal Lactobacilli has long been associated with increased risk for adverse birth outcomes including preterm birth. A recent Cochrane review concluded that while antibiotics can eradicate BV, it did not reduce the risk of a subsequent preterm birth [50].

Vaginal microbiotas dominated by L. crispatus (CST I) tends to be the most stable and to promote community stability [3]. Observational studies have shown the presence of L. crispatus in the vaginal microbiota is associated with the absence of BV [3]. Preterm birth was shown to be significantly associated with a lower frequency of L. crispatus, whereas the frequency of G. vaginalis was associated with BV and preterm birth [51]. The ratio of L. crispatus to Gardnerella revealed a strong exclusionary interaction. In contrast, exclusion between L. iners and Gardnerella was weak or absent as both were observed to often coexist at near equal frequencies [51]. In another study, L. crispatus dominance was highly predictive of term birth, although dominance of Lactobacillus species occurred in equal proportions of women who experienced preterm or term birth [52].

PROM is often unexpected and is responsible for approximately 30% of all preterm births [53]. MIAC is associated with ~30% of cases, and following PROM, 60-70% will have evidence of histological intra-amniotic infection (IAI) [54-57]. The pro-inflammatory response to MIAC can be detrimental to the fetus through the fetal inflammatory response syndrome [58].

In women with singleton pregnancies complicated by PROM, those with vaginal microbiotas dominated by L. crispatus (CST I) had a low rate of MIAC characterized by a low intra-amniotic microbial load and inflammation without IAI. Those with low vaginal Lactobacilli abundance or absence (CST IV) had significantly higher rates of IAC and IAI with pronounced intra-amniotic inflammation [59].

L. crispatus-dominated vaginal communities (CST I) have a lower pH than communities dominated by other species [16]. Elevation in vaginal pH above 4.5 is a feature of BV. This displaces L. crispatus, but not L. iners, which has adapted to up-regulate genes for carbohydrate metabolism. L. iners is one of the most commonly observed vaginal microbial species reported from both healthy and BV-diagnosed women, in contrast with L. crispatus which is mainly isolated from healthy women [4].

Again, production of the D-lactic acid isomer by some Lactobacillus strains is protective against microbial invasion of the upper genital tract. Matrix metalloproteinase 8 (MMP-8) decreases the integrity of the cervical os and can permit passage of microbes into the uterine cavity [60]. Elevated levels of MMP-8 in amniotic fluid is a risk factor for spontaneous preterm delivery [61]. Vaginal epithelial cells produce extracellular MMP inducer (EMMPRIN), the inducer of MMP-8, and its concentration in vaginal secretions are dependent on relative levels of D- and L-lactic acid [14]. High D-lactic acid production limits EMMPRIN concentrations, thus MMP-8 levels. Concentrations of the D-lactic acid isomer in vaginal secretions are highest with L. crispatus dominance [14], but L. iners-dominated women have lower concentrations of D-lactic acid, which may contribute to the higher prevalence of BV and preterm birth in these women.

Taken together, research evidence clearly suggests the prevalence of L. crispatus in the vaginal microbiota is an indicator of vaginal microbial ecosystem balance and health. Since maintenance of a healthy vaginal microbiota and vaginal acidity could reduce the incidence of BV/urogenital infections and adverse pregnancy outcomes, prophylactic supplementation with probiotics validated to promote vaginal Lactobacilli abundance and acidity is warranted, particularly in women with increased risk.

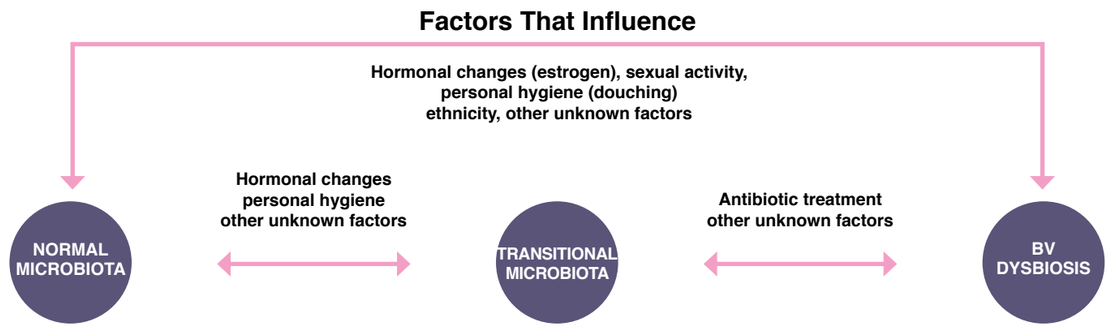

Fig 5. Common factors that influence the composition of the vaginal microbiota. Adapted from M. Petrova *et al. Trends in Microbiology* 2017

Vaginal Probiotics

Maintenance of a healthy vaginal microbiota could reduce the incidence of urogenital infections, the spread of sexually transmitted infections, and adverse pregnancy outcomes, thus decrease the need for conventional antimicrobial treatments [44].

Strains Matter

Bacterial migration from the colon to the vagina occurs naturally, thus is a source of both pathogens and Lactobacilli. Certain Lactobacillus strains can safely colonize the vagina after oral or vaginal administration, displace and kill pathogens, and modulate host immune responses.

These activities are generally strain specific; therefore, not all strains of a given Lactobacillus species have the same probiotic potential to support women's urogenital tract health. Per the definition of probiotics, a health benefit to the host, humans in our case, must be realized for a given condition and demonstrated to be superior to that of placebo in clinical research. Thus, specific strains indicated as probiotics to support women's urogenital tract health require validation of efficacy in human studies, but the vast majority of products claiming probiotics for this indication have not been clinically validated.

Selection Criteria

Domig et al. [45] demonstrated an extensive, multi-step scientific process to identify candidate probiotic strains, from the predominant Lactobacillus species colonizing the vagina of healthy pregnant women, for oral administration to support women's urogenital tract health. Strains L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 were selected for targeted formulation based on relevant criteria:

- Ability to grow under aerobic and anaerobic conditions
- Acidification capacity
- Glycogen utilization
- Extracellular hydrogen peroxide production
- Stability under acidic conditions and resistance to bile salts (important for survival during gastrointestinal transit)
- Anti-microbial activity against multiple strains of common vaginal pathogens (i.e., Candida albicans, Candida krusei, Candida glabrata, E. coli, and G. vaginalis)
- Compatibility & Safety (e.g., lack of virulence factors, antibiotic susceptibility/lack of antibiotic resistance)
- Encapsulated stability as a multi-strain formulation (forecasting shelf-life for use in commercial dietary supplements)

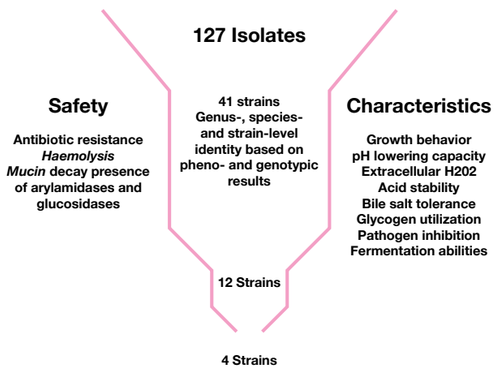

Fig 6. Strategy for selection of potential vaginal probiotic strains. Adapted from K.J. Domig *et al. Beneficial Microbes* 2014

These strains as part of multi-strain probiotic formulation were subsequently demonstrated in multiple clinical studies to increase vaginal Lactobacilli abundance and acidification in support of urogenital tract health.

Adjuvant Therapy for Bacterial Vaginosis

Yogurt containing live cultures, including Lactobacilli (e.g., starter cultures L. bulgaricus and S. thermophilus), is often touted as a natural remedy for bacterial vaginosis (BV), despite lack of scientific support. Fermented foods containing live cultures are often mistaken for probiotics, but a fermented food is not a source of probiotics unless it contains strain(s) that satisfy the criteria for a probiotic, particularly for a condition beyond general digestive health support.
In a randomized, double-blind, placebo-controlled clinical trial, oral supplementation with L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 significantly improved cure rate and symptoms of BV [46]. Women with newly diagnosed BV (based on Amsel criteria, diagnostic criteria for BV of which 3 of 4 criteria must be met: pH > 4.5, positive whiff test, presence of discharge, and presence of clue cells in the wet smear) were given metronidazole for 1-week and the multi-strain vaginal probiotics daily in yogurt (n = 17) or acidified yogurt (n = 17). After a 4-week intervention period, 0/17 women supplemented with the vaginal probiotics had BV compared to 6/17 (35%) women given only yogurt. Amsel score was reduced by 4 vs. only 2, along with significantly reduced odor and discharge, in women supplemented with vaginal probiotics compared to those given yogurt only.

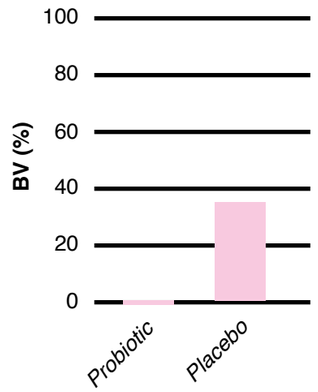

Fig 7. Women with bacterial vaginosis (BV). Adapted from C. Laue *et al. Beneficial Microbes* 2017

Vaginal pH in Postmenopausal Women with Breast Cancer

Given the positive influence of estrogen on vaginal Lactobacilli, the vaginal microbiota of postmenopausal women is generally characterized by decreased vaginal Lactobacilli and increased vaginal pH. Clinical symptoms often experienced include vaginal dryness, burning, itching, painful intercourse, painful urination, urinary frequency, and recurrent urinary tract infections (UTIs) [47, 48].

Urogenital symptoms of menopause are especially common in women with breast cancer due to chemotherapy and estrogen deprivation therapy. Vaginal estrogen therapy increases vaginal Lactobacilli and decreases colonization by uropathogenic E. coli to reduce UTI recurrence [49], but estrogen therapy for women with breast cancer is used with restraint due to frequent estrogen sensitivity of the tumor [50].

In a randomized, double-blind, placebo-controlled clinical study, daily oral supplementation with L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 (5 billion viable cells) for 2 weeks in postmenopausal women with breast cancer receiving chemotherapy, with vaginal atrophy and an intermediate vaginal microbiota (Nugent score 4 - 6) (n = 11), improved the Nugent score (-1.3) towards a normal microbiota (< 3), whereas there was a deterioration in women receiving placebo (+0.45).

Vaginal Microbiota in Immunosuppressed Pregnant Women

The mother is the main source of microbes for newborn colonization. Vaginal microbiota dysbiosis with reduced Lactobacilli increases risk of obstetric complications such as placental insufficiency, premature birth, fetal growth restriction, and postpartum endometritis [6, 38, 51, 52]. This is particularly relevant for immunosuppressed women with herpes virus infection (HVI).
L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N,

and L. rhamnosus LbV 96 were evaluated as a therapeutic and preventative intervention for pregnant women with HVI. Sixty pregnant women with HVI either received a patented food supplement containing the vaginal probiotic strains twice daily for 1-week (n = 30) or only prenatal care (n = 30).

Intestinal Lactobacilli and Bifidobacteria were significantly increased, and pathogenic microbes (hemolytic E. coli, Klebsiella pneumoniae, Staphylococcus aureus, Candida yeast species) were significantly decreased in women receiving the probiotics. This was accompanied by reduced symptoms of intestinal microbiota dysbiosis including bloating, abdominal discomfort, and constipation.

The percentage of women with vaginal Lactobacilli increased from 13.3% to 46.7% after 1-week supplementation of the probiotics, but was not increased in non-supplemented women. Also, the percentage of women with a vaginal pH > 4.5, complaining of vaginal discharge, swelling (hyperemia), itching (pruritus), and with a positive amine test of vaginal discharge, was significantly decreased post probiotic supplementation, but not changed in non-supplemented women.

The incidence of placental insufficiency and fetal distress were significantly reduced approximately 2-fold in the probiotic supplemented women compared to non-supplemented women. The percentage of women with other pregnancy complications (e.g., threatened miscarriage, threat of premature birth, pre-eclampsia, and pathology of amniotic fluid) was 25-50% lower in supplemented vs. non-supplemented women.

The Neovagina: A Challenging Microbial Environment

The neovaginal microbiota of male-to-female transsexual women is a diverse community with very limited colonization by Lactobacilli, more reflective of the abnormal vaginal microbiota characteristic of bacterial vaginosis (BV) [53]. The neovaginal environment may not adequately support the growth of Lactobacilli, with one study observing a neovaginal Lactobacilli colonization rate of only 4% [54].
In a prospective, randomized, placebo-controlled study, daily oral supplementation with L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 (5 billion live cells) for only 1-week significantly enriched Lactobacilli and resulted in a lower Nugent score in the neovagina of male-to-female transsexual women compared to placebo [55]. Neovaginal Lactobacilli abundance was 5-6 times higher in the intervention group compared to the placebo group. In those with a Nugent score indicative of BV (> 7), an improvement in the Nugent score was observed after the 1-week oral probiotic supplementation, but did not improve in those receiving placebo.

Summary

The application of true probiotics to support women's urogenital tract health (i.e., clinically validated dosing of probiotic formulations such as L. crispatus LbV 88 + L. jensenii LbV 116 + L. gasseri LbV 150N + L. rhamnosus LbV 96) in clinical practice has gained increasing recognition as a therapy to help restore and maintain vaginal microbial ecosystem balance. Utilization during pregnancy to reinforce vaginal Lactobacilli abundance and stability within this dynamic microbial community is likely warranted to reduce risk of adverse pregnancy outcomes associated with vaginal microbiota dysbiosis. Furthermore, prophylactic and adjuvant use to anti-microbial therapies has the potential to improve efficacy and reduce prescription, including inappropriate, of anti-microbials to attenuate the spread of resistance as one of the world's most pressing public health problems.

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FAQs

Q1: Are these Lactobacilli strains genetically modified organisms (GMOs)?

A: No. L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 were isolated from healthy pregnant women and selected from multiple strains of these predominant Lactobacillus species for probiotic attributes to support vaginal and urinary tract health.

Q2: Is there any benefit to using these probiotic strains if I am already healthy?

A: Yes. The vaginal microbiota is dynamic and its composition can change rapidly in response to various common biological, environmental, and behavioral factors. Furthermore, many otherwise healthy women may still have low vaginal Lactobacilli abundance. Reduced protective vaginal Lactobacilli can increase risk of various adverse gynecological and obstetric outcomes. Use of probiotic strains clinically validated to support vaginal microbial ecosystem balance promotes health maintenance.

Q3: How long will it take to see effects of supplementation with these probiotic strains?

A: Clinical research has shown increased vaginal Lactobacilli in as little as one week of oral supplementation with L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96.

Q4: Are there any side effects or reasons women should not use these probiotic strains?

A: Increased incidence of side effects relative to placebo have not been observed in clinical studies nor has there been any reports of side effects with use of these probiotic strains. However, a women should consult with her doctor if being cared for due to preexisting illness.

Q5: Should I use these probiotic strains every day?

A: Probiotics used to promote health should be used daily since probiotics do not reside in or on us long after we discontinue use. Probiotics exert their benefits as they transit and transiently colonize body surfaces.

Q6: Can I supplement with these probiotic strains during/after doctor-prescribed antibiotic treatment?

A: Yes, in fact, it is recommended to help maintain and restore protective vaginal Lactobacilli, which can be diminished by antibiotic use.

Q7: Can I direct apply these probiotic strains (i.e., vaginal suppository)?

A: Efficacy and safety of L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 have been demonstrated with oral use. Although these strains have not been directly administered vaginally in clinical research.

Q8: Is it okay to use these probiotic strains during pregnancy?

A: Yes, in fact, it is recommended during this vulnerable period. Loss of vaginal Lactobacilli increases risk of an imbalanced vaginal microbial ecosystem and urogenital infection, which can lead to adverse obstetric outcomes such as miscarriage or preterm birth.

Q9: Does oral supplementation with these strains benefit gastrointestinal health?

A: Oral supplementation with L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 was shown to increase friendly intestinal bacteria (i.e., Lactobacilli and Bifidobacteria), reduce multiple undesirable intestinal bacteria and yeast species, and reduce symptoms (e.g., bloating, abdominal discomfort, constipation) in pregnant women with compromised immune function and dysbiosis of the intestinal microbiota, which suggests these strains also benefit gastrointestinal health with oral use.

Q10: Can these probiotic strains alone be used as a treatment for a yeast infection?

A: No, or at least it has not been shown in clinical research, but it is anticipated to augment recovery during conventional treatment due to its ability to restore and maintain vaginal Lactobacilli and acidity, which discourages yeast overgrowth. L. crispatus LbV 88, L. jensenii LbV 116, L. gasseri LbV 150N, and L. rhamnosus LbV 96 have been shown to antagonize growth of Candida yeast species in vitro.

Vaginal Microbiota

by Anthony P. Thomas, PhD

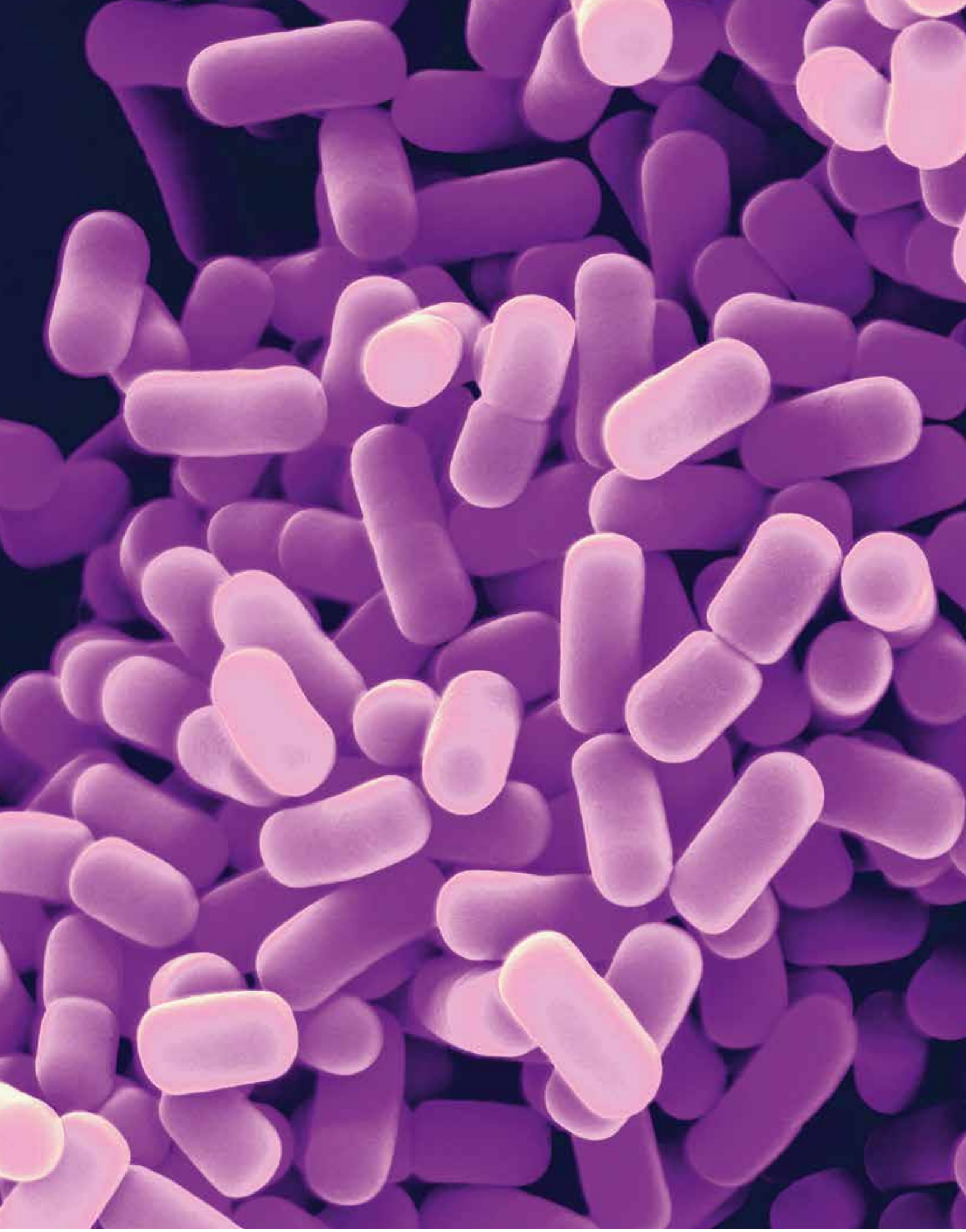
Rationale and Clinical Research of Vaginally-Derived Probiotic Strains to Support Female Urogenital Tract Health

Bio

Anthony Thomas, Ph.D. earned his B.A. in Nutrition, Food Science, and Dietetics from California State University Northridge, his doctorate in Nutritional Biology from the University of California at Davis, and conducted postdoctoral research at the University of California at Los Angeles Larry Hillblom Islet Research Center.

His primary research interests (via both pre-clinical and clinical studies) have focused on the influence of dietary and lifestyle factors (i.e., physical activity, circadian disruption) on the pathogenesis of chronic cardiovascular/metabolic diseases including obesity, insulin resistance syndrome, and type 2 diabetes.

He has authored/co-authored multiple peer reviewed scientific manuscripts and has served as a referee with relevant expertise in the fields of nutrition, obesity, and diabetes for multiple scientific journals.



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