Exploring New Treatments for Bacterial Vaginosis: Evaluating the Synergistic Effects of Prebiotics and Lactic Acid on *Lactobacillus Crispatus* growth

Lehan Gu, Elizabeth Krider and Manjula Gunawardana

Abstract

Background: Bacterial vaginosis (BV) is a common vaginal condition caused by an imbalance in the microbiome. *Lactobacillus crispatus* plays a key role in vaginal health by producing lactic acid, which lowers pH and inhibits growth of bacteria causing BV. Prebiotic and postbiotic compounds such as D-mannose, glycogen, inulin and lactic acid might help restore a healthy vaginal microenvironment by stimulating growth of *L. crispatus*.

Methods: Concentrations of D-mannose, glycogen, inulin, and lactic acid for enhancing *L. crispatus* were screened. Growth curves of *L. crispatus* in the presence and absence of these compounds (1–10 mg/ml, LMRS media) were obtained from 16-hour incubations, and biofilm assays were performed on select concentrations.

Results: Specific prebiotic and postbiotic enhancers can significantly promote the growth of *L. crispatus* in liquid media when added in the concentration range of 1-6 mg/ml. Inulin (I) was the most efficient enhancer (31-56% relative enhancement) of the three prebiotics. The combination of D-mannose (M) and glycogen (G) had the strongest relative enhancement (152%). Adding D-lactic acid (DLA) in a triple combination increased growth rates across all combinations, with G+I+DLA mix yielding the highest enhancement (162%). Biofilms data showed dramatic enhancements for the following combinations: 208% for M+G+DLA, 177% for G+I+DLA, and 144% for M+I+DLA.

Conclusions: This preliminary study highlights the potential of enhancer supplementation to stimulate regrowth of *L. crispatus*, which could be the foundation of a viable strategy in the treatment of recurrent BV.

Keywords:
Bacterial vaginosis, biofilms, *Lactobacillus crispatus*, prebiotics, probiotics, postbiotics, glycogen, inulin, D-mannose, lactic acid.

Introduction

Bacterial vaginosis (BV) is a common vaginal condition caused by an imbalance in the microbiome. Though typically treated with antibiotics, BV-associated bacteria (BVAB) can easily form biofilms and develop antibiotic resistance, which leads to the frequent regrowth of harmful bacteria and prolonged BV. *Lactobacillus crispatus* plays a key role in vaginal health by producing lactic acid, which lowers pH and inhibits BVAB growth. Bacterial vaginosis (BV) is caused by an imbalance in the vagina, characterized by symptoms like fishy odor, vaginal discharge, itching, and pain during urination. There are multiple reproductive health issues associated with BV, including a high risk of contracting and spreading sexually transmitted infections (STIs), like HIV, and pregnancy complications[1]. It is caused by a disruption of the healthy vaginal microbiota, typically marked by a decline in protective *Lactobacilli* and the

overgrowth of BV-associated bacteria (BVAB), such as *Gardnerella vaginalis*, *Mobiluncus sp.*, and *Prevotella sp.*

Vaginal microbiota are characterized by community state types (CSTs), consisting of *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus gasseri*, and *Lactobacillus jensenii*[2]. *L. crispatus* plays a fundamental protective role in vaginal health[3–5]. Its ability to produce D- and L-lactic acid, which lowers vaginal pH to a range of 3.8-4.5 and inhibits BVAB growth, distinguishes it among the six CSTs[6]. The hydrogen peroxide produced by *L. crispatus* also promotes vaginal health by lowering specific vaginal pro-inflammatory cytokines, aiding the immunomodulation in the vagina and providing reproductive health benefits[7].

Common treatment for BV is topical, suppository or oral antibiotics such as metronidazole or clindamycin. These antibiotics can ameliorate the symptoms at around 70–85% of women with BV within 1 month[8]. However, BVAB can easily form sticky biofilms on the vaginal epithelium that prevent antibiotics from penetrating the biofilm and targeting the bacterial cells. Therefore, recurrence is a severe problem in BV treatment, evidenced by an over-half recurrence rate after 12 months, as reported in a study in 2006[9]. Furthermore, women often express dissatisfaction with frequent antibiotic use.

Alternative Treatments for BV: Enhancers + *L. crispatus*

Researchers are currently investigating the role of “prebiotics” to enhance the growth of key bacterial species, wherein prebiotics are defined as non-digestible substances that promote the growth of specific bacteria that reside in the human body. This is in contrast to the term “probiotics,” which refers to the beneficial living microorganisms inhabiting the gut, respiratory passages, or vagina. “Postbiotics” are defined as active substances produced by probiotics and help maintain the stability and health of the localized bacterial community otherwise known as a microbiome[10].

One example of the “prebiotics” or “enhancer” strategy is the development of a live biotherapeutic product called LACTIN-V, which includes *L. crispatus* CTV-5[11]. Clinical trials have demonstrated that intravaginal administration of LACTIN-V following antibiotic treatment significantly reduces BV recurrence by promoting colonization of *L. crispatus*[11]. As of 2025, LACTIN-V is in phase 2/phase 3 clinical trials for BV and UTI treatment, with ongoing FDA evaluation. Recent studies have also explored the use of postbiotic gels containing lactic acid and hyaluronic acid to alleviate BV symptoms[12]. These gels have been shown to improve vaginal pH and reduce inflammation, creating a more favorable environment for beneficial bacteria[12]. To further support the regrowth of *L. crispatus* in the vaginal environment, researchers are now investigating the inclusion of small molecules in vaginal suppositories.

Possible candidates include compounds such as D-mannose, inulin, glycogen, and D-lactic acid because of their roles in bacterial growth. D-mannose, a simple monosaccharide, is best known for its ability to prevent pathogenic bacterial adhesion in urinary tract health. *E. coli* is a bacterium that often causes UTIs. FimH is a bacterial adhesion protein located on the tip of *E.coli* that binds to the surface of host epithelial cells. By binding to FimH, D-mannose blocks the binding between FimH and epithelial cells, allowing the bacteria to be flushed out with urine[13]. While no studies have shown that BV is directly related to *E.coli*, D-mannose has been proven to attenuate the conditions of multiple UTIs, making it a good choice when testing prebiotics for BV treatment[14].

Glycogen is a primary energy source for *L. crispatus* that resides in the vaginal epithelial cells and is metabolized under anaerobic conditions[15]. Once secreted into the vaginal lumen during the lysis of epithelial cells, it is cleaved by enzymes like amylopullulanases and other

extracellular glycoside hydrolases[16,17]. Then, simpler sugars like maltose and maltotriose produced by this hydrolysis are metabolized by *L. crispatus*, leading to lactic acid production, which in turn lowers the vaginal pH. Additionally, it serves as the food source for BVAB as well, and the consumption of glycogen by BVAB is faster than Lactobacillus, resulting in a depletion of food source for the latter in the context of BV[18]. Consequently, adding glycogen as a prebiotic helps restore a healthy vaginal microbiome balance by inhibiting BVAB growth and supplementing *L. crispatus* food source.

Inulin is a plant-derived fructooligosaccharide that is widely accepted as a prebiotic for some gut bacteria in gastrointestinal research[19]. These studies have shown that *Lactobacillus* can express inulinase that breaks down inulin into simple sugars, which are fermented to produce short-chain fatty acids (SCFAs) and lactic acid[20]. However, there are limited studies on inulin's effect on promoting *L. crispatus* regrowth. Still, the extensive evidence of inulin's efficacy in the GI system motivated us to investigate its potential effects in the vaginal environment.

Lactic acid plays a central role in vaginal health by creating an acidic environment and inhibiting the growth of pathogenic bacteria. It is primarily produced by *Lactobacillus* species through the fermentation of glycogen-derived sugars. Specifically, glycolysis of these sugars produces pyruvates, which is then reduced to lactic acid.

It has two isomers, D-lactic acid and L-lactic acid. While both can help lower the pH and prevent BVAB from dominating the vaginal microenvironment, studies suggest that D-lactic acid plays a more critical protective role than L-lactic acid[5]. In BV, cervical-vaginal epithelial cells can easily be over-shredded, weakening its barrier function that normally prevents infection caused by the dominant BVAB[21]. In a study, higher abundance of bacterial lactate dehydrogenase (the enzyme that makes lactic acid) was positively correlated with the expression of epithelial barrier protein[22]. In contrast, when simply lower the pH while not using lactic acid, the result didn't replicate the high correlation. Together, they confirmed lactic acid's protective effect in the vagina.

Problem Statement and Rationale

Introducing *L. crispatus* via suppository into the vaginal space after a female has taken antibiotics to eliminate an episode of BV is one strategy under development by clinical researchers and scientists. Fledgling *L. crispatus* populations in the vagina may not outcompete BVAB and other pathogens, unless growth enhancers are included in the suppository formulation. In this context, questions arise: which small molecules might serve as prebiotics to stimulate *L. crispatus* growth and at what concentrations will such effects be observed? Moreover, studies reporting the impact of small molecules on *L. crispatus* growth have not evaluated possible growth synergies of the individual compounds.

Significance and Purpose

Women suffering from recurring BV are confronted by two major problems: (1) the rise in antibiotic resistance to standard BV treatments, and (2) the challenge of restoring *L. crispatus* as the dominant species in the vaginal microbiome. Clinicians and health professionals need a new type of treatment for BV. Delivering *L. crispatus* in the form of a vaginal suppository may hold promise if adjuvants known to support *L. crispatus* growth are incorporated into the suppository. Such a development would improve the treatment options for women worldwide who suffer from BV.

Objectives:

The main objectives of this study are:

- To characterize the effect of select compounds (enhancers) on the growth of *L. crispatus* in liquid cultures, including D-mannose (M), glycogen (G), inulin (I), D-lactic acid (DLA), and L-lactic acid (LLA).
- To evaluate the synergistic effects, if any, of specific combinations of these compounds on *L. crispatus* bacterial growth.
- To measure the effects of enhancers on biofilm formation of *L. crispatus* as measured by crystal violet assays.

Recognizing the importance of *L. crispatus* in maintaining vaginal health and the limitations of antibiotic therapy, this study aims to investigate whether supplementing *L. crispatus* cultures with selected prebiotic enhancers can effectively promote bacterial regrowth. We seek to characterize which enhancer might support a clinical product containing *L. crispatus* to be used for successful bacterial reestablishment in the vaginal environment. As an extension, we also investigated how the combination of different enhancers can further boost the *L. crispatus* regrowth. Thereby, this paper offers insights into potential adjuvant strategies to complement conventional BV treatment.

Scope and Limitations

This study screens the concentrations at which 5 enhancer candidates and several of their combinations might promote bacterial growth of *L. crispatus* in liquid culture and in biofilm assays. This work focuses on growth enhancement *in vitro*; it does not include any *in vivo* studies that might demonstrate the utility of such enhancers to promote *L. crispatus* restoration in animal models.

Methodology Overview

Experimental Design. Bacterial growth curves and biofilm studies were conducted with human-sourced *L. crispatus* in L-MRS media and incubated at 37 C. Individual compounds were added to the bacterial cultures which had been diluted in fresh media. Varying concentrations of D-mannose (D), glycogen (G), inulin (I), D-lactic acid (DLA), and L-lactic acid (LLA) were added to the cultures, and the growth rates over specific time periods were compared to determine the extent of growth enhancements conferred by the treatments. Representative concentrations for each individual enhancer were selected based on statistical confidence level and subsequently used in the combination studies that explored possible synergistic effects from the enhancer combinations. Biofilm studies using crystal violet were performed to assess the effects of added enhancers. Data were processed, analyzed and graphed to illustrate the influence of individual and synergistic combinations of enhancers on *L. crispatus* growth.

Materials and Methods

Media, Chemicals and Instrumentation

- **Media and Chemicals:** L-MRS (deMan, Rogosa, and Sharpe) media and all chemicals (glycogen, inulin, D-mannose, D-lactic acid, L-lactic acid, crystal violet) were purchased from Sigma.

- **Bacteria:** The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: *Lactobacillus crispatus*, Strain MV-3A-US, HM-636.
- **Analytical Instruments:** changes in optical density were measured in microtiter plates using a Molecular Devices SpectraMax 384 Microplate reader. Data were collected every hour, and the temperature of the plate chamber was maintained at 37 C.
- **Laboratory Equipment:** All microbiology work was conducted using aseptic technique in a Biosafety Level-2 cabinet with laminar flow capability. Standard laboratory tools such as an analytical balance, micropipettes, and filter sterilization (using 0.2 micron PTFE filters) were used throughout the studies.

Bacterial Preparation

Bacterial strains were cultured overnight in sterile L-MRS media anaerobically at 37 C and supplemented with 5% CO_2. Cultures were serially diluted by 1:5 in fresh media over a range of 1:5-1:3125, and the optical density at 600 nm was monitored for each dilution during an observation window of 0-16 hours (Figure 1). The bacterial dilution of 1:625 was used for all studies reported here.

Enhancer Preparation

Stock solutions (20-40 mg/mL) for each enhancer candidate were prepared in LMRS and filter-sterilized using 0.2 micron PTFE filters. Glycogen was used as received from the manufacturer as a suspension at 20 mg/ml. Stock solutions were further diluted with sterile LMRS media to give enhancer solutions (8-40 mg/mL).

Bacterial Growth Studies

Diluted cultures (150 uL) were delivered via multichannel pipette or individually in triplicate to a 96-well microtiter plate. Fifty microliters (50 uL) from the diluted enhancer stock solutions (8-40 mg/mL) were delivered to the corresponding wells to achieve final concentrations of 1-10 mg/mL. For the control group (bacteria only), 50 uL of sterile media was added.

Crystal Violet Biofilm Assays

Diluted cultures (150 uL) were delivered via multichannel pipette or individually in triplicate to a 96-well microtiter plate. Fifty microliters (50 uL) from the diluted enhancer stock solutions (8-40 mg/mL) were delivered to the corresponding wells to achieve final concentrations of 1-10 mg/mL. For the control group (bacteria only), 50 uL of sterile media was added. The plates were incubated at 37 C for 24 hours in anaerobic conditions. The planktonic cells were carefully discarded by pipetting, and the wells were washed gently with 200 ul of PBS two times. Two hundred microliters of 0.1% crystal violet solution was added to each well, and the plate was left undisturbed for 30 minutes at room temperature. The stain was removed and the wells were washed three times with sterile distilled water. The plate was inverted and allowed to dry overnight at room temperature. Two hundred microliters of 30% (v/v) acetic acid was added to each well to solubilize the bound dye. The plate was incubated for 10-15 minutes at room temperature with gentle shaking. The absorbance at 595 nm was measured using the microplate reader. The mean absorbance of negative control wells was subtracted from the

experimental wells to correct for background effects, and the results were expressed as a mean from the technical replicates (n = 3).

Results

Optimal Bacterial Dilution.

Because *L. crispatus* inoculum concentration influences growth potential, we first determined the optimal bacterial dilution in the absence of enhancers. Among the tested dilutions (5 - 3125X), 5X is the most concentrated solution and demonstrated a typical bacterial growth curve during an overnight incubation. The increase in optical density of 1 full unit during 14 hours reflects the most rapid growth among the dilutions tested. In contrast, the other dilutions (25X–3125X) exhibited slower or minimal growth over this same time period; the media only group remained unchanged, confirming that there were no contaminations. As a result, 5X was chosen as the dilution factor used in the enhancer studies because the positive effects of added enhancers will likely be pronounced on this time scale.

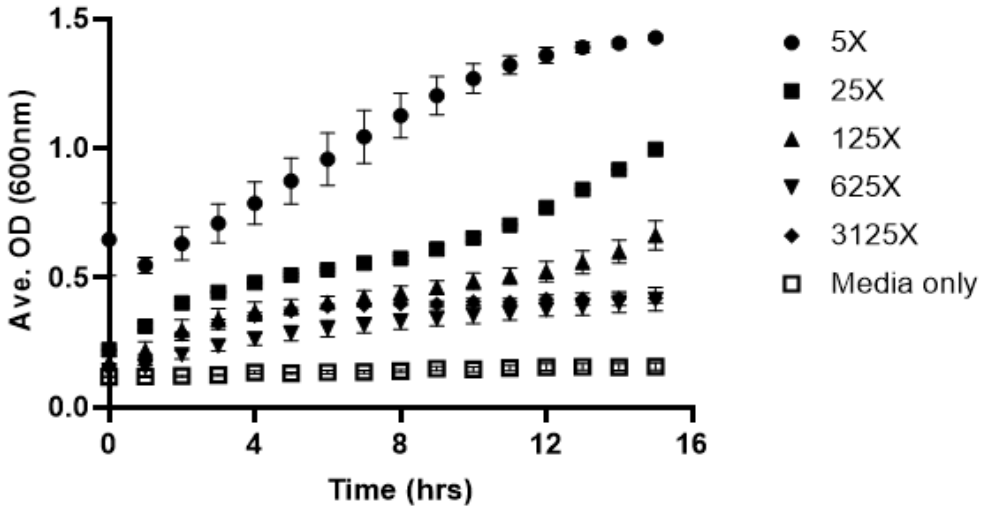

Figure 1: changes in optical density (600 nm) over time for *L. crispatus* cultures in LMRS media incubated in a temperature-controlled microplate reader. Five bacterial dilutions were evaluated to identify concentrations suitable for overnight studies.

The effect of enhancers on *L. crispatus* growth

To distinguish the magnitude of any enhancements when enhancers are added at different concentrations, the slope of the growth curves was calculated for the time period of t=5 hours to t=10 hours. Described below are the results obtained when D-mannose (M), glycogen (G), and inulin (I) incubated individually with *L. crispatus* cultures for 16 hours. Combinations of enhancers were prepared and the resulting growth enhancements were calculated for liquid culture growth assays and biofilm assays.

D-Mannose (M). To evaluate whether D-mannose (M) affects *L. crispatus* growth, bacteria were grown in media and incubated with M at 2-10 mg/ml, alongside the bacteria-only control group. Figure 2 shows a comparison of the growth rates between 5-10 hours of the 16-hour incubation period at different concentrations of M, relative to the control group. The smallest relative

enhancement effect (25%) was observed at 2 mg/ml M. The relative enhancements for 4 mg/ml (51%), 6 mg/ml (43%), 8 mg/ml (41%) and 10 mg/ml (52%) M appear to be comparable to one another, given the error bars for each result. This effect does not seem to be dose-dependent.

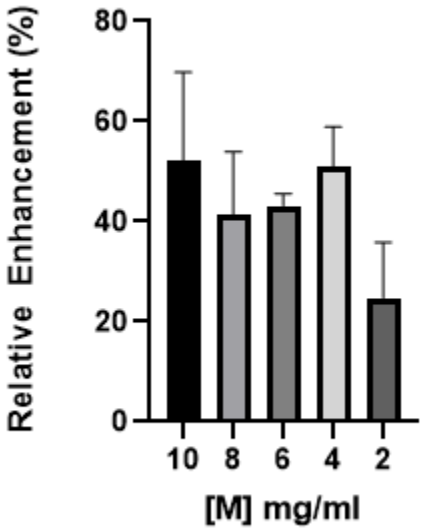

Figure 2. The relative enhancements in *L. crispatus* growth rates at different concentrations of D-mannose (M), as calculated for growth between 5-10 hours of a 16-hour incubation period.

Glycogen (G). Different concentrations of glycogen (1-5 mg/ml) were introduced to *L. crispatus* cultures, and the relative enhancements in growth were observed to be similar to each other at these concentrations as shown in Figure 3: 34% (1 mg/ml), 43% (2 mg/ml), 37% (3 mg/ml), 35% (4 mg/ml), and 34% (5 mg/ml). No dose-response effect was observed in this concentration range.

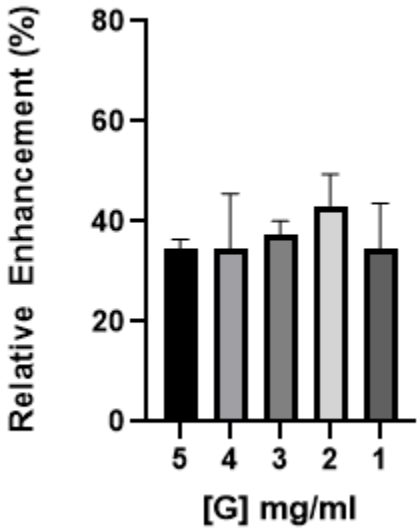

Figure 3. The relative enhancements in *L. crispatus* growth rates at different concentrations of glycogen (G), as calculated for growth between 5-10 hours of a 16-hour incubation period.

Inulin (I). Different concentrations (1-5 mg/ml) of inulin (I) were added individually to the *L. crispatus* cultures (Figure 4). The relative enhancements in growth are similar in scale over this concentration range: 31% (1 mg/ml), 49% (2 mg/ml), 43% (3 mg/ml), 54% (4 mg/ml), and 56% (5 mg/ml). A modest dose-dependent increase in relative enhancement was observed in this concentration range.

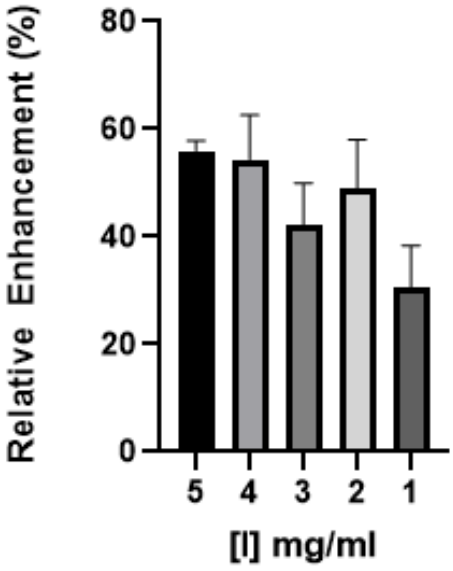

Figure 4. The relative enhancements in *L. crispatus* growth rates at different concentrations of inulin (I), as calculated for growth between 5-10 hours of a 16-hour incubation period.

Lactic Acid (DLA, LLA). To evaluate the effect of exogenous D- and L-lactic acid on *L. crispatus* growth, bacteria were incubated with DLA and LLA at 2-10 mg/ml, in addition to the bacteria-only control group. The average growth rates were calculated at each concentration of DLA and LLA. The average growth rates (optical density per hour) over 16 hours at these concentrations are shown in Figure 5. The L isomer of lactic acid shows no effect on growth rates, whereas the D isomer of lactic acid shows a measurable effect compared to the cells-only cultures. D-lactic acid was used in subsequent combination experiments in our study.

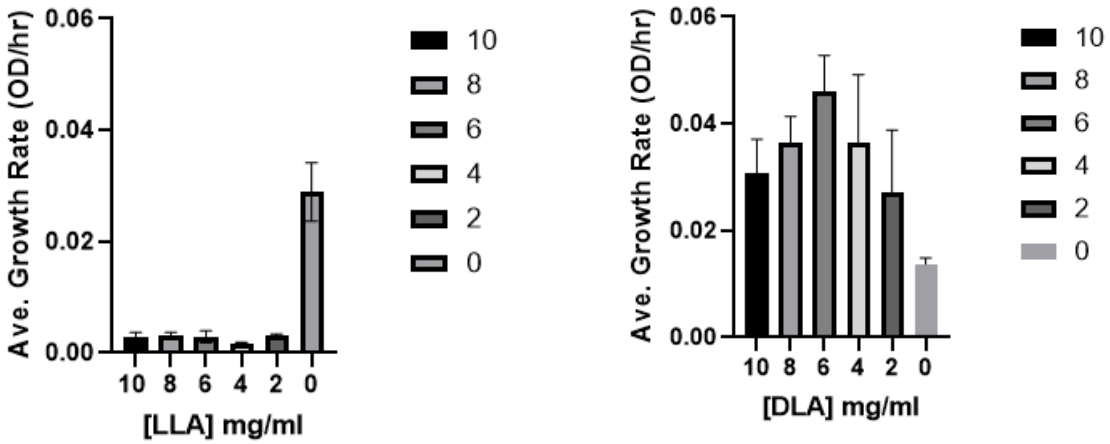

Figure 5. The average growth rates of *L. crispatus* cultures in the presence of (a) f L-lactic acid (LLA) and (b) D-lactic acid (DLA) over a range of concentrations (2-10 mg/ml) during a 16-hour incubation period.

Enhancer combinations

Possible synergistic effects on relative enhancement were determined when two enhancers were added together at their representative concentrations. On a scale of 0-250% relative enhancement, all three combinations shown in Figure 6 result in positive enhancement. The greatest enhancement (152%) was observed when M (6 mg/ml) and G (3 mg/ml) were combined, whereas the combination of G (3 mg/ml) and I (2 mg/ml) had the least enhancement effect of 55%.

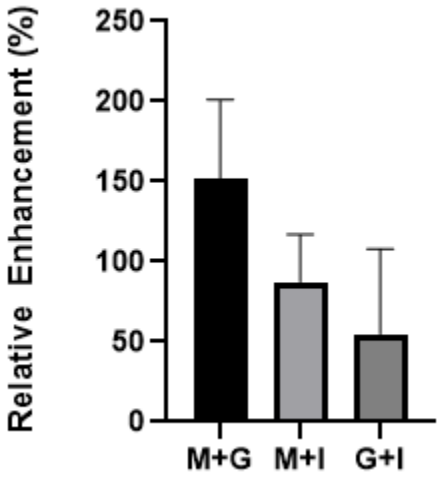

Figure 6. The relative enhancement of enhancer combinations on *L. crispatus* growth between 5-10 hours of a 16-hour incubation period.

To probe the effect of three-enhancer combinations, DLA (6 mg/ml) was added to the two-enhancer groups. All combinations produced higher relative enhancements compared to groups without DLA (Figure 7). Among the tested conditions, the G + I + DLA group yielded the greatest effect, with a relative enhancement of 163%, while the M + I + DLA group yielded the smallest effect at 109%. Moreover, the G + I + DLA group had a statistically significant difference from the other two combinations, indicating a solid stronger enhancement effect. However, the M + G + DLA (126%) and M + I + DLA (109%) groups have a similar relative enhancement due to their overlapping error bars, similar to the pattern observed from the previously tested groups.

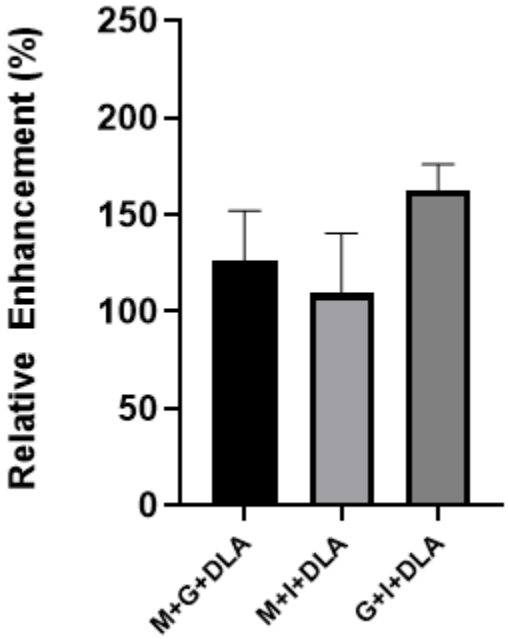

Figure 7. The relative enhancement in *L. crispatus* growth between t=5 hours and t=10 hours of incubation with triple combinations of enhancers (D-mannose, M; glycogen, G; inulin, I; D-lactic acid, DLA).

Biofilms Assay

To explore the effects of enhancers on *L. crispatus* biofilm formation, the enhancers were added individually and in combinations. Figure 8 shows the relative enhancement in biofilm formation for each enhancer and combination thereof. Inulin (109%, 2 mg/ml) enhanced biofilm formation to a greater degree than D-mannose (32%, 6 mg/ml) and glycogen (30%, 3 mg/ml) when enhancers were added individually to the *L. crispatus* culture. When combined in pairs, the relative enhancements in biofilm formation showed no additive effect for M+G (35%) but a synergistic effect for the M+I group (89%) and G+I group (113%). Adding D-lactic acid to the enhancer pairs resulted in significant overall enhancement compared to the single and paired enhancer groups: 209% for M+G+DLA, 144% for M+I+DLA, and 177% for G+I+DLA.

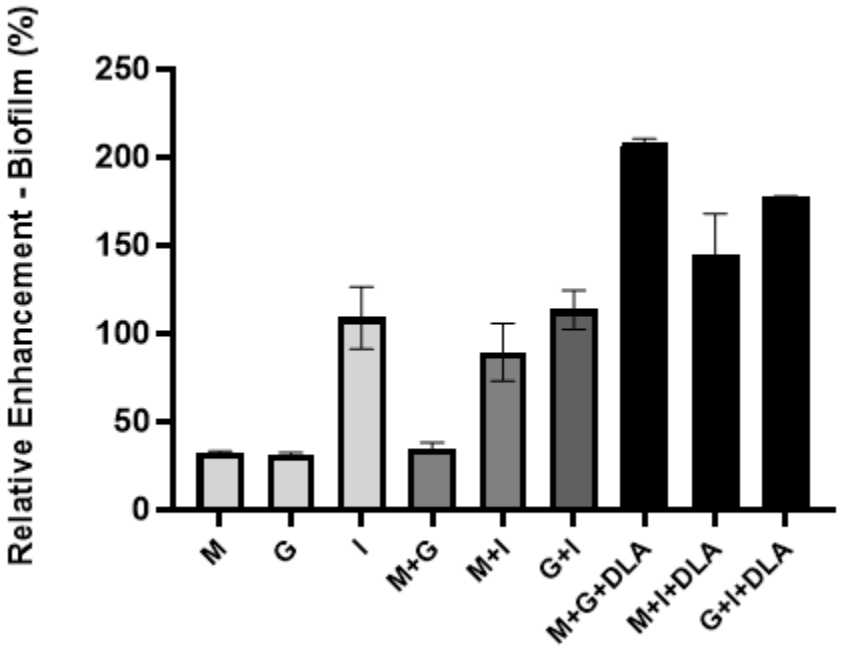

Figure 8. The relative enhancement in biofilm formation of *L.crispatus* in the presence of different enhancers after a 24-hour incubation period.

Discussion

Restatement of Key Findings

Our study shows that specific prebiotic and postbiotic enhancers can significantly promote the growth of *L. crispatus* in liquid media. When added individually, inulin was the most efficient enhancer (31-56% relative enhancement) followed by D-mannose (25-52% relative enhancement) and Glycogen (34-43%relative enhancement), depending on the concentrations

used. When enhancers were combined at their representative concentrations, the combination of D-mannose and glycogen had the strongest relative enhancement (152%), while combinations with inulin were less effective. Importantly, adding D-lactic acid in a triple combination, increased growth rates across all combinations, with G+I+DLA mix yielding the highest enhancement (162%).

The crystal violet assay data showed the effects on biofilm formation that the enhancers contributed at their representative concentrations and in combination with each other. When added individually, inulin (2 mg/ml) produced the highest relative enhancements at 109%. When added in combination, the strongest enhancement was observed for cultures containing inulin: 89% for M+I and 113% for G+I. The addition of D-lactic acid (DLA) to each double combination showed dramatic enhancements in biofilm formation: 208% for M+G+DLA, 177% for G+I+DLA, and 144% for M+I+DLA.

Implications and Significance

There is significant research on the interaction of the chosen enhancers and microbiota residing in the human body. Previous studies have shown that glycogen serves as a food source for vaginal *Lactobacillu*s species. However, most of these studies focused on its role in triggering lactic acid secretion instead of the direct growth stimulation of *L. crispatus.* Similarly, D-mannose primarily acts as a uropathogenic *E. coli* adhesion blocker, so its effect of serving as a growth substrate for *L. crispatus* is limited. There is also little indication that it can promote *L. crispatus* biofilm formation[23]. Although inulin has been widely studied as a gastrointestinal prebiotic, there is a lack of data on its impact within the vaginal microbiome. Finally, lactic acid isomers are known to contribute to lowering vaginal pH thus preventing BVAB biofilm formation, yet few studies have investigated their synergistic role for growth stimulation when combined with prebiotics. Our study addresses these gaps by providing a systematic comparative analysis of D-mannose, glycogen and inulin, with D-lactic acid added for additional growth promotion.

Connection to Objectives

This study successfully met its main objectives. First, the ability of D-mannose, glycogen, inulin, and D-lactic acid to promote the growth of *L. crispatus* was observed, with each enhancer and each combination showing a difference in the enhancement magnitude. L-lactic acid did not enhance the growth rate of *L. crispatus* at the concentrations screened. Second, the examination of how these substances work together demonstrates that combinations like glycogen with D-mannose and glycogen with inulin and D-lactic acid were effective. While not all possible combinations were explored, the data indicate clear synergy from the enhancers at the representative concentrations used. Finally, biofilm assays confirmed the effectiveness of certain enhancers for stimulating *L. crispatus* growth.

Recommendations

Future research should focus on expanding biofilm assays to explore a broader range of enhancer combinations and concentrations. In addition, more detailed comparisons between conditions like D-mannose + glycogen + D-lactic acid (D + G + DLA) and glycogen + inulin + D-lactic acid (G + I + DLA) are needed. Our current data cannot fully differentiate which of these two groups can better enhance the growth due to a highly similar relative enhancement value and the error bars. Higher-resolution studies would help clarify the most synergistic formulations

and identify dose-dependent effects more clearly. In this way, our study provides a foundation for future work.

From a practical standpoint, future studies could move beyond *in vitro* screening toward *in vivo* validation, with pH level examined to verify whether the formation of *L. crispatus* biofilm with enhancers in it lowers the vaginal pH to a degree that restores the microbiome balance. Expanding these findings into tissue testing, animal models, and eventually clinical studies will also be crucial for establishing safety and efficacy in application settings.

Limitations

These results were generated through *in vitro* experiments, which do not fully replicate the complexity of the vaginal environment. It does not capture important factors like host immune interactions, epithelial glycogen metabolism, and competing bacterial species. Future research should expand to include *in vivo* models, dose optimization, and more detailed studies to explain how each enhancer affects *L. crispatus* physiology.

Conclusion

Since antibiotics often fail to prevent BV recurrence, many women are frustrated by repeated cycles of treatment. Therefore, the four enhancers tested in this study for their ability to stimulate growth of *L. crispatus* might offer a promising alternative treatment. Giving patients hope and confidence in treatments that are effective, widely accessible and affordable fulfills the promise of fundamental research.

References

1. Cohen CR, Lingappa JR, Baeten JM, et al. Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Analysis among African Couples. *PLOS Medicine*. 2012;**9(6)**:e1001251. doi:10.1371/journal.pmed.1001251

2. De Seta F, Campisciano G, Zanotta N, Ricci G, Comar M. The Vaginal Community State Types Microbiome-Immune Network as Key Factor for Bacterial Vaginosis and Aerobic Vaginitis. *Front Microbiol*. 2019;**10**:2451. doi:10.3389/fmicb.2019.02451

3. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microb Cell Fact*. 2020;**19(1)**:203. doi:10.1186/s12934-020-01464-4

4. Breshears LM, Edwards VL, Ravel J, Peterson ML. Lactobacillus crispatus inhibits growth of Gardnerella vaginalis and Neisseria gonorrhoeae on a porcine vaginal mucosa model. *BMC Microbiology*. 2015;**15(1)**:276. doi:10.1186/s12866-015-0608-0

5. France M, Alizadeh M, Brown S, Ma B, Ravel J. Towards a deeper understanding of the vaginal microbiota. *Nature Microbiology*. 2022;**7(3)**:367-378. doi:10.1038/s41564-022-01083-2

6. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. *Research in Microbiology*. 2017;**168(9)**:782-792. doi:10.1016/j.resmic.2017.04.001

7. Mitchell C, Fredricks D, Agnew K, Hitti J. Hydrogen-peroxide producing lactobacilli are associated with lower levels of vaginal IL1β, independent of bacterial vaginosis. *Sex Transm Dis*. 2015;**42(7)**:358-363. doi:10.1097/OLQ.0000000000000298

8. Vodstrcil LA, Muzny CA, Plummer EL, Sobel JD, Bradshaw CS. Bacterial vaginosis: drivers of recurrence and challenges and opportunities in partner treatment. *BMC Medicine*. 2021;**19(1)**:194. doi:10.1186/s12916-021-02077-3

9. Bradshaw CS, Morton AN, Hocking J, et al. High Recurrence Rates of Bacterial Vaginosis over the Course of 12 Months after Oral Metronidazole Therapy and Factors Associated with Recurrence. *The Journal of Infectious Diseases*. 2006;**193(11)**:1478-1486. doi:10.1086/503780

10. Ji J, Jin W, Liu S, Jiao Z, Li X. Probiotics, prebiotics, and postbiotics in health and disease. *MedComm* (2020). 2023;**4(6)**:e420. doi:10.1002/mco2.420

11. Cohen CR, Wierzbicki MR, French AL, et al. Randomized Trial of Lactin-V to Prevent Recurrence of Bacterial Vaginosis. *New England Journal of Medicine*. 2020;**382(20)**:1906-1915. doi:10.1056/NEJMoa1915254

12. Shen X, Xu L, Zhang Z, et al. Postbiotic gel relieves clinical symptoms of bacterial vaginitis by regulating the vaginal microbiota. *Front Cell Infect Microbiol.* 2023;**13**. doi:10.3389/fcimb.2023.1114364

13. Wagenlehner F, Lorenz H, Ewald O, Gerke P. Why d-Mannose May Be as Efficient as Antibiotics in the Treatment of Acute Uncomplicated Lower Urinary Tract Infections—Preliminary Considerations and Conclusions from a Non-Interventional Study. *Antibiotics (Basel)*. 2022;**11(3)**:314. doi:10.3390/antibiotics11030314

14. Caretto M, Giannini A, Russo E, Simoncini T. Preventing urinary tract infections after menopause without antibiotics. *Maturitas.* 2017;**99**:43-46. doi:10.1016/j.maturitas.2017.02.004

15. Hertzberger R, May A, Kramer G, van Vondelen I, Molenaar D, Kort R. Genetic Elements Orchestrating Lactobacillus crispatus Glycogen Metabolism in the Vagina. *Int J Mol Sci.* 2022;**23(10)**:5590. doi:10.3390/ijms23105590

16. Navarro S, Abla H, Delgado B, Colmer-Hamood JA, Ventolini G, Hamood AN. Glycogen availability and pH variation in a medium simulating vaginal fluid influence the growth of vaginal Lactobacillus species and Gardnerella vaginalis. *BMC Microbiology*. 2023;**23(1)**:186. doi:10.1186/s12866-023-02916-8

17. Jenkins DJ, Woolston BM, Hood-Pishchany MI, et al. Bacterial amylases enable glycogen degradation by the vaginal microbiome. *Nat Microbiol.* 2023;**8(9)**:1641-1652. doi:10.1038/s41564-023-01447-2

18. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic Lactobacillus species in vaginal health. *Research in Microbiology.* 2017;**168(9)**:782-792. doi:10.1016/j.resmic.2017.04.001

19. Jolliff JS, Mahan DC. Effect of Dietary Inulin and Phytase on Mineral Digestibility and Tissue Retention in Weanling and Growing Swine1,2. *J Anim Sci.* 2012;**90(9)**:3012-3022. doi:10.2527/jas.2011-4424

20. Wu XZ, Wen ZG, Hua JL. Effects of dietary inclusion of Lactobacillus and inulin on growth performance, gut microbiota, nutrient utilization, and immune parameters in broilers. *Poultry Science.* 2019;**98(10)**:4656-4663. doi:10.3382/ps/pez166

21. O'Hanlon DE, Gajer P, Brotman RM, Ravel J. Asymptomatic Bacterial Vaginosis Is Associated With Depletion of Mature Superficial Cells Shed From the Vaginal Epithelium. *Front Cell Infect Microbiol.* 2020;**10**:106. doi:10.3389/fcimb.2020.00106

22. Delgado-Diaz DJ, Jesaveluk B, Hayward JA, et al. Lactic acid from vaginal microbiota enhances cervicovaginal epithelial barrier integrity by promoting tight junction protein expression. *Microbiome.* 2022;**10(1)**:141. doi:10.1186/s40168-022-01337-5

23. Giordani B, Naldi M, Croatti V, et al. Exopolysaccharides from vaginal lactobacilli modulate microbial biofilms. *Microbial Cell Factories.* 2023;**22(1)**:45. doi:10.1186/s12934-023-02053-x